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Serial No.: **09/875,221** Group No.: **1646**

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For: **BIOLOGICAL PRODUCTS**

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On December 18, 2001

Doreen Yatko Trujillo

Doreen Yatko Trujillo, Reg. No. 35,719

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Attached please find the certified copy of the foreign application from which priority is claimed for this case:

Country: United Kingdom

Application No: 0013810.7

Filing Date: June 6, 2000

Applicant respectfully requests that receipt of the enclosed document be acknowledged and that the U.S. Patent Office records be updated accordingly.

Respectfully submitted,

Date: December 18, 2001

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1. Your reference — **P021741GB: CPM/AGT**

2. Patent application number *(The Patent Office will fill in this part)* — **0013810.7**

6 JUN 2000

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)* 07907579001

If the applicant is a corporate body, give the country/state of its incorporation — **UNITED KINGDOM**

4. Title of the invention — **BIOLOGICAL PRODUCTS**

5. Name of your agent *(if you have one)*

Carpmaels & Ransford

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Signature: Carpmaels & Ransford.

Date: **6th June 2000**

Carpmaels & Ransford

12. Name and daytime telephone number of person to contact in the United Kingdom: **MERCER, CHRISTOPHER PAUL 020-7242 8692**

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Biological Products

The present invention relates to an antibody molecule having specificity for antigenic determinants of tumour necrosis factor alpha (TNFα). The present invention also relates to the therapeutic uses of the antibody molecule and methods for producing the antibody molecule.

Construction of CDR-grafted antibodies is described in European Patent Application EP-A-0239400, wherein the CDRs of a mouse monoclonal antibody have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The CDRs determine the antigen binding specificity of antibodies and are relatively short peptide sequences carried on the framework regions of the variable domains. There are three CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

The earliest work on humanising monoclonal antibodies by CDR-grafting was carried out on monoclonal antibodies recognising synthetic antigens, such as NP or NIP antigens. However, examples in which a mouse monoclonal antibody recognising lysozyme and a rat monoclonal antibody recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen *et al* (Science, 239, 1534-1536, 1988) and Riechmann *et al* (Nature, 332, 323-324, 1988), respectively.

In Riechmann *et al*, it was found that the transfer of the CDR regions alone (as defined by Kabat (Kabat *et al*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA; and Wu *et al*, J. Exp. Med., 132, 211-250, 1970)) was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. It was found that a number of framework residues have to be altered so that they correspond to those of the donor framework region. Proposed criteria for selecting which framework residues need to be altered are described in International Patent Application WO 90/07861.

A number of reviews discussing CDR-grafted antibodies have been published including Vaughan *et al* (Nature Biotechnology, 16, 535-539, 1998).

TNFα is a proinflammatory cytokine that is released by and interacts with cells of the immune system. Thus, TNFα is released by macrophages that have been activated by lipopolysaccharides (LPS) of gram negative bacteria. As such, TNFα appears to be an endogenous mediator of central importance involved in the development and pathogenesis

of endotoxic shock associated with bacterial sepsis. TNFα has also been shown to be upregulated in a number of human diseases, including chronic diseases such as rheumatoid arthritis, Crohn's disease, ulcerative colitis and multiple sclerosis. TNFα is therefore referred to as a pro-inflammatory cytokine.

Monoclonal antibodies against TNFα have been described in the prior art. Meager *et al.*, (Hybridoma, 6, 305-311, 1987) describe murine monoclonal antibodies against recombinant TNFα. Fendly *et al.*, (Hybridoma, 6, 359-370, 1987) describe the use of murine monoclonal antibodies against recombinant TNFα in defining neutralising epitopes on TNF. Shimamoto *et al.*, (Immunology Letters, 17, 311-318, 1988) describe the use of murine monoclonal antibodies against TNFγ and their use in preventing endotoxic shock in mice. Furthermore, in International Patent Application WO 92/11383, recombinant antibodies, including CDR-grafted antibodies, specific for TNFα are disclosed. Rankin *et al.*, (British J. Rheumatology, 34, 334-342, 1995) describe the use of such CDR-grafted antibodies in the treatment of rheumatoid arthritis. United States patent US-A-5,919,452 discloses anti-TNF chimeric antibodies and their use in treating pathologies associated with the presence of TNF.

Antibodies to TNFα have been proposed for the prophylaxis and treatment of endotoxic shock (Beutler *et al*, Science, 234, 470-474, 1985). Bodmer *et* al, (Critical Care Medicine, 21, S441-S446, 1993) and Wherry *et al*, (Critical Care Medicine, 21, S436-S440, 1993) discuss the therapeutic potential of anti-TNFα antibodies in the treatment of septic shock. The use of anti-TNFα antibodies in the treatment of septic shock is also discussed by Kirschenbaum *et al*, (Critical Care Medicine, 26, 1625-1626, 1998). The use of anti-TNFα antibodies in the treatment of rheumatoid arthritis and Crohn's disease is discussed in Feldman *et al*, (Transplantation Proceedings, 30, 4126-4127, 1998), Adorini *et al*, (Trends in Immunology Today, 18, 209-211, 1997) and in Feldman *et al*, (Advances in Immunology, 64, 283-350, 1997). The antibodies to TNFα used in such treatments are generally chimeric antibodies such as those described in US-A-5,919,452.

The prior art anti-TNFα antibody molecules generally have a reduced affinity for TNFα compared to the antibodies from which the variable regions or CDRs are derived, generally have to be produced in mammalian cells and are expensive to manufacture.

There is a need for an antibody molecule to treat chronic inflammatory diseases that can be used repeatedly and produced easily and efficiently. There is also a need for an antibody molecule which has high affinity for TNFα and low immunogenicity in humans.

In a first aspect, the present invention provides an antibody molecule having specificity for TNFα, comprising a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat *et al*, 1987, *supra*) having the sequence given in SEQ ID NO:1 for CDR1, SEQ ID NO:2 for CDR2 or SEQ ID NO:3 for CDR3.

In a second aspect of the present invention, there is provided an antibody molecule having specificity for human TNFα, comprising a light chain wherein the variable domain comprises a CDR (as defined by Kabat *et al*, 1987, *supra*) having the sequence given in SEQ ID NO:4 for CDR1, SEQ ID NO:5 for CDR2 or SEQ ID NO:6 for CDR3.

Preferably, the antibody molecule of the first and second aspects of the present invention has a complementary light chain or a complementary heavy chain, respectively.

In a third aspect of the present invention there is provided an antibody molecule having specificity for human TNFα, comprising a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat *et al*, 1987, *supra*) having the sequence given in SEQ ID NO:1 for CDR1, SEQ ID NO:2 for CDR2 or SEQ ID NO:3 for CDR3, and a light chain wherein the variable domain comprises a CDR (as defined by Kabat *et al*, 1987, *supra*) having the sequence given in SEQ ID NO:4 for CDR1, SEQ ID NO:5 for CDR2 or SEQ ID NO:6 for CDR3.

The CDRs given in SEQ ID NOS:1 to 6 referred to above are derived from a mouse monoclonal antibody hTNF40. However, SEQ ID NO:2 consists of a hybrid CDR. The hybrid CDR comprises part of a CDR from mouse monoclonal antibody hTNF40 (SEQ ID NO: 7) and part of a CDR from a human group 3 germline H2 CDR sequence. The complete sequences of the variable domains of the hTNF40 antibody are shown in Figures 6 (light chain) and Figure 7 (heavy chain). The mouse antibody is referred to below as "the donor antibody".

The present invention also provides the mouse monoclonal antibody hTNF40. The sequences of the variable domains of the light and heavy chains of hTNF40 are shown in Figures 6 and 7, respectively. The light chain constant region of hTNF40 is Kappa and the heavy chain constant region is IgG2a.

The present invention also provides a chimeric mouse/human antibody molecule referred to herein as the chimeric hTNF40 antibody molecule. The chimeric antibody

molecule comprises the variable domains of the mouse monoclonal antibody hTNF40 and human constant domains. Preferably the chimeric hTNF40 antibody molecule comprises the human C kappa domain (Hieter *et al.*, Cell, 22, 197-207, 1980; Genebank accession number J00241) and the human gamma 4 domain (Flanagan *et al.*, Nature, 300, 709-713, 1982).

Preferably the antibody molecule of the present invention is a CDR-grafted antibody molecule. The term "a CDR-grafted antibody molecule" as used herein refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs from a donor antibody (e.g. a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody).

Preferably, the antibody molecule according to any one of the aspects of the present invention has a variable domain comprising a human acceptor framework and one or more of the donor CDRs referred to above.

In a preferred embodiment of the present application, the donor antibody is the hTNF40 murine anti-human TNFα antibody, and the acceptor antibody is a human antibody.

The antibody molecule of the first or third aspect of the present invention comprises at least one CDR selected from SEQ ID NOS: 1 to 3 for the heavy chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the heavy chain variable domain.

The antibody molecule of the second or third aspect of the present invention comprises at least one CDR selected from SEQ ID NOS: 4 to 6 for the light chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the light chain variable domain.

The CDRs of the heavy chain variable domain are located at residues 26-35 (CDR1), residues 50-65 (CDR2) and residues 95-102 (CDR3) according to the Kabat numbering.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR1), residues 50-56 (CDR2), and residues 89-97 (CDR3) according to the Kabat numbering.

Residue designations given above and elsewhere in this present application are according to the Kabat numbering (Kabat *et al*, 1987, *supra*). Thus, the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino

acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

When the CDRs are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which CDRs are derived, including mouse, primate and human framework regions. Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al*, 1987, *supra*). For example, KOL and NEWM can be used for the heavy chain and REI can be used for the light chain and EU, LAY and POM for both the heavy chain and the light chain. The preferred framework regions for the light chain are the human group 1 consensus framework regions shown in Figure 1. The preferred framework regions for the heavy chain are the human group 1 and group 3 consensus framework regions shown in Figure 2.

The present invention also provides a CDR-grafted antibody molecule according to the first or third aspect of the present invention wherein the acceptor framework regions of the heavy chain comprise donor residues at positions 27, 68, 70, 72, 73 and 76 (according to Kabat *et al*, 1987, *supra*) when the donor has the human group 1 or group 3 consensus framework regions shown in Figure 2. Donor residues are residues from the donor antibody i.e. the antibody from which the CDRs were originally derived.

The present invention also provides a CDR-grafted antibody molecule according to the second or third aspect of the present invention wherein the acceptor framework regions of the light chain comprise donor residues at positions 46 and 60 (according to Kabat *et al*, 1987, *supra*) when the donor is the human group 1 consensus framework regions shown in Figure 1.

The antibody molecule of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, Fab', $F(ab')_2$ or Fv fragment; a light chain or heavy chain monomer or dimer; a single chain antibody, e.g. a single chain Fv in which heavy and light chain variable domains are joined by a peptide linker; or any CDR-grafted molecule with the same specificity as the original donor antibody. Similarly, the heavy and light chain variable regions may be combined with other antibody domains as appropriate. Preferably the antibody molecule of the

present invention is a Fab fragment. It is further preferred that the Fab fragment is modified to allow the attachment of PEG groups.

Also, the antibody molecule of the present invention may have an effector or a reporter molecule attached. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, procedures of recombinant DNA technology may be used to produce an antibody molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by or has attached thereto by peptide linkage a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

The antibody molecule of the present invention preferably has a binding affinity of at least $0.5x10^{10}\ M^{-1}$, more preferably at least $0.75x10^{10}\ M^{-1}$ and most preferably at least $0.85x10^{10}\ M^{-1}$.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simply blocking TNFα activity.

The remainder of the antibody molecule need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

Preferably, the antibody molecule of the present invention comprises the light chain variable domain hTNF40-gL1 and the heavy chain variable domain gh3hTNF40.4. The sequence of the variable regions of these light and heavy chain variable regions are shown in Figures 8 and 11, respectively.

The present invention also relates to variants of the antibody molecule of the present invention, which have an improved affinity for TNF . Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al*, J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks *et al.*, Bio/Technology, 10, 779-783,

1992), use of mutator strains of *E. coli* (Low *et al.*, J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten *et al.*, Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson *et al.*, J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Crameri *et al.*, Nature, 391, 288-291, 1998). Vaughan *et al.* (*supra*) discusses these methods of affinity maturation.

The antibody molecule of the present invention may be PEGylated (i.e. have PEG (polyethylene glycol) covalently attached) according to the method disclosed in EP-A-0 948 544. Preferably the antibody molecule is PEGylated by the attachment of the molecule shown in Figure 13.

The present invention also provides a PEGylated antibody molecule according to the present invention.

The present invention also provides a DNA sequence encoding the antibody molecule of the present invention or the heavy and/or light chain of the antibody molecule.

Preferably, the DNA sequence encodes the heavy and/or light chain of the antibody molecule of the present invention. In a preferred embodiment, the DNA sequence encodes the light chain of the antibody molecule of the present invention and comprises the sequence shown in SEQ ID NO: 8 or 10 encoding the variable region of the light chain of an antibody molecule of the present invention. In a further preferred embodiment, the DNA sequence encodes the heavy chain of the antibody molecule of the present invention and comprises the sequence shown in SEQ ID NO: 12 or 14 encoding the variable region of the heavy chain of an antibody molecule of the present invention.

The DNA sequence of the present invention may comprise synthetic DNA, cDNA or genomic DNA, or any combination thereof.

The present invention also relates to a cloning or expression vector comprising the DNA sequence of the present invention. Preferably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, respectively.

In a preferred embodiment, the present invention provides an *E. coli* expression vector comprising the DNA sequence of the present invention. Preferably the expression vector is pTTO as shown schematically in Figure 21.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art.

DNA sequences which encode the antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used, in part, for expression of antibody fragments such as Fab and $F(ab')_2$ fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eucaryotic, e.g. mammalian, host cell expression systems may be used for production of larger antibody molecules, including complete antibody molecules. Suitable mammalian host cells include CHO cells or myeloma or hybridoma cell lines.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell comprising a vector of the present invention under conditions suitable for leading to expression of DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

Preferably the process for the production of the antibody molecule of the present invention comprises culturing *E. coli* comprising an *E. coli* expression vector comprising the DNA sequence of the present invention under conditions suitable for leading to expression of the DNA sequence and isolating the antibody molecule. The antibody molecule may be secreted from the cell or targeted to the periplasm by suitable signal sequences. Alternatively, the antibody molecules may accumulate within the cell's cytoplasm. Preferably the antibody molecule is targeted to the periplasm. Depending on the antibody molecule being produced and the process used, it is desirable to allow the

antibody molecule to refold and form a functional confirmation. Procedures for allowing antibody molecule to refold are well known to those skilled in the art.

The antibody molecule may comprise only heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

The present invention also provides a therapeutic or diagnostic composition comprising the -grafted antibody molecule of the present invention in combination with a pharmaceutically acceptable excipient, diluent or carrier.

The present invention also provides a process for preparation of a therapeutic or diagnostic composition comprising admixing the antibody molecule of the present invention together with a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the therapeutic or diagnostic composition or may be accompanied by one or other active ingredients including other antibody ingredients, for example, anti-T cell, anti-IFNγ or anti-LPS antibodies, or non-antibody ingredients such as xanthines. The therapeutic and diagnostic compositions may be in unit dosage form, in which case each unit dose comprises an effective amount of the antibody molecule of the present invention. An effective dose of the antibody of the present invention is preferably between 0.5 and 50mg/kg, more preferably between 1 and 20mg/kg and most preferably about 15mg/kg.

The present invention also provides the antibody molecule of the present invention for use in treating a disease mediated by TNFα.

The present invention also provides the use of the antibody molecule according to the present invention in the manufacture of a medicament for the treatment of a disease mediated by TNFα.

The antibody molecule of the present invention may be utilised in any therapy where it is desired to reduce the level of TNFα present in the human or animal body. The TNFα may be circulating in the body or present in an undesirably high level localised at a particular site in the body.

For example, elevated levels of TNFα are implicated in acute and chronic immune and immunoregulatory disorders, infections including septic, endotoxic and cardiovascular shock, inflammatory disorders, neurodegenerative diseases, malignant diseases and alcohol induced hepatitis. Details of the numerous disorders associated with elevated levels of TNFα are set out in US-A-5,919,452. The antibody molecule of the present invention may be utilised in the therapy of diseases mediated by TNFα. Particularly preferred diseases which may be treated by the antibody molecule of the present invention include sepsis, septic or endotoxic shock, cachexia, adult respiratory distress syndrome, AIDS, allergies, psoriasis, T.B., inflammatory bone disorders, blood coagulation disorders, burns, rejection episodes following organ or tissue transplant, Crohn's disease and autoimmune diseases, such as thyroiditis and rheumatoid- and osteo-arthritis.

Additionally, the antibody molecule or composition may be used: to reduce side effects associated with TNFα generation during neoplastic therapy; to eliminate or reduce shock related symptoms associated with the treatment or prevention of graft rejection by use of an anti-lymphocyte antibody; or for treating multi-organ failure.

The antibody molecule of the present invention is preferably for treatment of rheumatoid- or osteo-arthritis.

The antibody molecule of the present invention may be administered in any appropriate form and amount according to the therapy in which it is employed.

Suitable forms for administration include forms suitable for parenteral administration e.g. by injection or infusion, for example, by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents.

Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

If the antibody molecule is suitable for oral administration, for example in the case of antibody fragments, the formulation may contain, in addition to the active ingredient, suitable additives used in the formulation of orally administered compositions.

The present invention also provides a method of treating human or animal subjects suffering from or at risk of a disorder mediated by TNFα, the method comprising administering to the subject an effective amount of the antibody molecule of the present invention.

The dose at which the antibody molecule of the present invention is administered depends on the nature of the condition to be treated, the degree to which the level of TNFα to be neutralised is, or is expected to be, raised above a desirable level, and on whether the antibody molecule is being used prophylactically or to treat an existing condition. The dose will also be selected according to the age and condition of the patient.

Thus, for example, where the product is for treatment or prophylaxis of a chronic inflammatory disease such as rheumatoid arthritis, suitable doses of the antibody molecule of the present invention lie in the range of between 0.5 and 50mg/kg, more preferably between 1 and 20mg/kg and most preferably about 15mg/kg. The frequency of doses will depend on the half live of the antibody molecule.

If the antibody molecule has a short half live (e.g. 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long half life (e.g. 2 to 15 days) it may only be necessary to give a dosage once per day, per week or even once every 1 or 2 months.

The antibody molecule of the present invention may also be used in diagnosis, for example, in the *in vivo* diagnosis and imaging of disease states involving elevated levels of TNFα.

The present invention also provides an antibody molecule comprising a hybrid CDR comprising a truncated donor CDR sequence wherein the truncated portion of the donor CDR is replaced by a different sequence and forms a functional CDR. The term "a hybrid CDR" as used herein means a CDR comprising a donor CDR which has been truncated at one or more positions, for example at one or both of its ends. The truncated portion of the donor CDR (i.e. the portion of the CDR which is missing) is replaced by a different sequence to form a complete and functional CDR. The hybrid CDR has at least one amino acid change compared to the complete donor CDR. The sequence replacing the truncated portion of the CDR can be any sequence. Preferably the sequence is from an antibody having the same framework regions as the antibody molecule of the present invention such as a germline antibody having consensus framework regions.

It has been found that antibody molecules comprising a hybrid CDR retain substantially the same binding affinity as an antibody molecule comprising complete donor CDRs. The term "substantially the same binding affinity" as used herein means at least 70%, more preferably at least 85% and most preferably at least 95% of the binding affinity

of the corresponding antibody molecule comprising complete donor CDRs. The use of a hybrid CDR provides the advantages of reducing the amount of foreign (i.e. donor) sequence present in the antibody molecule and can increase the binding affinity of the antibody molecule compared to the corresponding antibody molecule comprising complete donor CDRs.

Any of the CDRs of the antibody molecule can be hybrid. Preferably CDR2 of the heavy chain is hybrid in the antibody molecule.

Preferably the truncation of the donor CDR is from 1 to 8 amino acids, more preferably 4 to 6 amino acids. It is further preferred that the truncation is made at the C-terminus of the CDR.

Depending on the sequence of the truncated portion of the CDR and the sequence of the different sequence replacing the truncated portion, a number of amino acid changes may be made. Preferably at least 2 amino acid changes are made, more preferably at least 3 amino acid changes are made and most preferably at least 4 amino acid changes are made.

The present invention also provides a nucleic acid sequence which encodes the antibody molecule comprising a hybrid CDR of the present invention.

The present invention also provides an expression vector containing the nucleic acid sequence encoding the antibody molecule comprising a hybrid CDR of the present invention.

The present invention also provides a host cell transformed with the vector of the present invention.

The present invention also provides a process for the production of an antibody molecule comprising a hybrid CDR comprising culturing the host cell of the present invention and isolating the antibody molecule.

The present invention is further described by way of illustration only in the following examples which refer to the accompanying Figures.

Figure 1 shows the framework regions of the human light chain subgroup 1 compared to the framework regions of the hTNF40 light chain.

Figure 2 shows the framework regions of the human heavy chain subgroup 1 and subgroup 3 compared to the framework regions of the hTNF40 heavy chain.

Figure 3 shows the amino acid sequence of the CDRs of the hTNF40. CDR H2' is a hybrid CDR wherein the C-terminal six amino acids are from the H2 CDR sequence of a human subgroup 3 germline antibody. The amino acid changes to the sequence resulting from this hybridisation are underlined.

Figure 4 shows vector pMR15.1.

Figure 5 shows vector pMR14.

Figure 6 shows the nucleotide and predicted amino acid sequence of the murine hTNF40Vl.

Figure 7 shows the nucleotide and predicted amino acid sequence of the murine hTNF40Vh.

Figure 8 shows the nucleotide and predicted amino acid sequence of hTNF40-gL1.

Figure 9 shows the nucleotide and predicted amino acid sequence of hTNF40-gL2.

Figure 10 shows the nucleotide and predicted amino acid sequence of gh1hTNF40.4.

Figure 11 shows the nucleotide and predicted amino acid sequence of gh3hTNF40.4.

Figure 12 shows vector CTIL5-gL6.

Figure 13 shows the structure of the PEG molecule attached to the hTNF40 antibody molecule CDP870 (comprising hTNF40-gL1 and gh3hTNF40.4). n is 420.

Figure 14 shows vector pTTQ9.

Figure 15 shows the sequence of the OmpA oligonucleotide adapter.

Figure 16 shows vector pACYC184.

Figure 17 shows vector pTTO-1

Figure 18 shows vector pTTO-2.

Figure 19 shows vector pDNAbEng-G1.

Figure 20 shows the oligonucleotide cassettes encoding different intergenic sequences for *E. coli* Fab' expression.

Figure 21 shows periplasmic Fab' accumulation of IGS variants.

Figure 22 shows vector pTTO(CDP870).

EXAMPLES

Gene Cloning and Expression of a Chimeric hTNF40 Antibody Molecule

RNA Preparation from hTNF40 Hybridoma Cells

Total RNA was prepared from 3 x 10^7 hTNF40 hybridoma cells as described below. Cells were washed in physiological saline and dissolved in RNAzol (0.2 ml per 10^6 cells). Chloroform (0.2 ml per 2 ml homogenate) was added, the mixture shaken vigorously for 15 seconds and then left on ice for 15 minutes. The resulting aqueous and organic phases were separated by centrifugation for 15 minutes in an Eppendorf centrifuge and RNA precipitated from the aqueous phase by the addition of an equal volume of isopropanol. After 15 minutes on ice, the RNA was pelleted by centrifugation, washed with 70% ethanol, dried and dissolved in sterile, RNase free water. The yield of RNA was 400 μg.

PCR Cloning of hTNF40 Vh and Vl

cDNA sequences coding for the variable regions of hTNF40 heavy and light chains were synthesised using reverse transcriptase to produce single stranded cDNA copies of the mRNA present in the total RNA, followed by Polymerase Chain Reaction (PCR) on the cDNAs with specific oligonucleotide primers.

a) cDNA Synthesis

cDNA was synthesised in a 20 µl reaction containing the following reagents : 50mM Tris-HCl pH8.3, 75 mM KCl, 10mM dithiothreitol, 3 mM $MgCl_2$, 0.5 mM each deoxyribonucleoside triphosphates, 20 units RNAsin, 75 ng random hexanucleotide primer, 2 µg hTNF40 RNA and 200 units Moloney Murine Leukemia Virus reverse transcriptase. After incubation at 42°C for 60 minutes the reaction was terminated by heating at 95°C for 5 minutes.

b) PCR

Aliquots of the cDNA were subjected to PCR using combinations of primers for the heavy and light chains. The nucleotide sequences of the 5' primers for the heavy and light chains are shown in Tables 1 and 2 respectively. These sequences, all of which contain a restriction site starting 6 nucleotides from their 5' ends, followed by the sequence GCCGCCACC to allow optimal translation of the resulting mRNAs, an initiator codon and a further 20-30 nucleotides, are a compilation based on the leader peptide sequences of known mouse antibodies (Kabat *et al.*, Sequences of proteins of immunological interest 5th Edition 1991, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health).

The 3' primers are shown in Table 3. The light chain primer spans the V-C junction of the antibody and contains a restriction site for the enzyme SplI to facilitate cloning of the V1 PCR fragment. The heavy chain 3' primers are a mixture designed to span the J-C junction of the antibody. The first 23 nucleotides are identical to those found at the start of human C – gamma 1, 2, 3 and 4 genes and include the ApaI restriction site common to these human isotypes. The 3' region of the primers contains a mixed sequence based on those found in known mouse antibodies (Kabat *et al.*, 1991, *supra*).

The combinations of primers described above enables the PCR products for Vh and V1 to be cloned directly into the appropriate expression vector (see below) to produce chimeric (mouse-human) heavy and light chains and for these genes to be expressed in mammalian cells to produce chimeric antibodies of the desired isotype.

Incubations (100 µl) for the PCR were set up as follows. Each reaction contained 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 10 pmoles 5' primer mix (Table 4), 10 pmoles 3' primer (CL12 (light chain) or R2155 (heavy chain) (Table 3)), 1 µl cDNA and 1 unit Taq polymerase. Reactions were incubated at 95°C for 5 minutes and then cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, aliquots of each

reaction were analysed by electrophoresis on an agorose gel. Light chain reactions containing 5' primer mixes from light chain pools 1, 2 and 7 produced bands with sizes consistent with full length Vl fragments while reaction from the heavy chain reaction pool 3 produced a fragment with a size expected of a Vh gene. The band produced by the light chain pool 1 primers was not followed up as previous results had shown that this band corresponds to a light chain pseudogene produced by the hybridoma cell. Only the band from light chain reaction pool 2 was followed up.

c) Molecular Cloning of the PCR Fragments

The DNA fragments produced in the light chain reaction pool 2 were digested with the enzymes BstBI and Sp1I, concentrated by ethanol precipitation, electrophoresed on a 1.4% agarose gel and DNA bands in the range of 400 base pairs recovered. These were cloned by ligation into the vector pMR15.1 (Figure 4) that had been restricted with BstBI and SpII. After ligation, mixtures were transformed into *E. coli* LM 1035 and plasmids from the resulting bacterial colonies screened for inserts by digestion with BstBI and Sp1I. Representatives with inserts from each ligation were analysed further by nucleotide sequencing.

In a similar manner, the DNA fragments produced in the heavy chain reaction pool 3 were digested with HindIII and ApaI and cloned into the vector pMR14 (Figure 5) that had been restricted with HindIII and ApaI. Again, representative plasmids containing inserts were analysed by nucleotide sequencing.

d) Nucleotide Sequence Analysis

Plasmid DNA from a number of isolates containing Vh inserts was sequenced using the primers R1053 (which primes in the 3' region of the HCMV promoter in pMR14) and R720 (which primes in the 5' region of human C – gamma 4 and allows sequencing through the DNA insert on pMR14). It was found that the nucleotide sequences of the Vh insert in a number of clones were identical, except for differences in the signal peptide and J regions. This indicated that the clones examined are independent isolates arising from the use of different primers from the mixture of oligonucleotides during the PCR stage. The determined nucleotide sequence and predicted amino acid sequence of hTNF40Vh are given in Figure 7.

To analyse the light chain clones, the sequence derived from priming with R1053 and R684 (which primes in the 5' region of human C-kappa and allows sequencing through the DNA insert on pMR15.1) was examined. The nucleotide sequence and predicted amino

acid sequence of the Vl genes arising from reactions in pool 2 were similarly analysed. Again it was found that the nucleotide sequences of the Vl insert in a number of clones were identical, except for differences in the signal peptide and J regions, indicating that the clones examined were independent isolates arising from the use of different primers from the mixture of oligonucleotides used during the PCR stage. The determined nucleotide sequence and predicted amino acid sequence of hTNF40V1 are given in Figure 6.

TABLE 1

Oligonucleotide primers for the 5' region of mouse heavy chains.

CH1 : 5'ATGAAATGCAGCTGGGTCAT(G,C)TTCTT3'
CH2 : 5'ATGGGATGGAGCT(A,G)TATCAT(C,G)(C,T)TCTT3'
CH3 : 5'ATGAAG(A,T)TGTGGTTAAACTGGGTTTT3'
CH4 : 5'ATG(G,A)ACTTTGGG(T,C)TCAGCTTG(G,A)T3'
CH5 : 5'ATGGACTCCAGGCTCAATTTAGTTTT3'
CH6 : 5'ATGGCTGTC(C,T)T(G,A)G(G,C)GCT(G,A)CTCTTCTG3'
CH7 : 5'ATGG(G,A)ATGGAGC(G,T)GG(G,A)TCTTT(A,C)TCTT3'
CH8 : 5'ATGAGAGTGCTGATTCTTTTGTG3'
CH9 : 5'ATGG(C,A)TTGGGTGTGGA(A,C)CTTGCTATT3'
CH10 : 5'ATGGGCAGACTTACATTCTCATTCCT3'
CH11 : 5'ATGGATTTTGGGCTGATTTTTTTTATTG3'
CH12 : 5'ATGATGGTGTTAAGTCTTCTGTACCT3'

Each of the above primers has the sequence 5'GCGCGCAAGCTTGCCGCCACC3' added to its 5' end.

TABLE 2

Oligonucleotide primers for the 5' region of mouse light chains.

CL1 : 5'ATGAAGTTGCCTGTTAGGCTGTTGGTGCT3'
CL2 : 5'ATGGAG(T,A)CAGACACACTCCTG(T,C)TATGGGT3'
CL3 : 5'ATGAGTGTGCTCACTCAGGTCCT3'
CL4 : 5'ATGAGG(G,A)CCCCTGCTCAG(A,T)TT(C,T)TTGG3'
CL5 : 5'ATGGATTT(T,A)CAGGTGCAGATT(T,A)TCAGCTT3'

CL5A : 5'ATGGATTT(T,A)CA(A,G)GTGCAGATT(T,A)TCAGCTT3'

CL6 : 5'ATGAGGT(T,G)C(T,C)(T,C)TG(T,C)T(G,C)AG(T,C)T(T,C)CTG(A,G)G3'

CL7 : 5'ATGGGC(T,A)TCAAGATGGAGTCACA3'

CL8 : 5'ATGTGGGGA(T,C)CT(G,T)TTT(T,C)C(A,C)(A,C)TTTTTCAAT3'

CL9 : 5'ATGGT(G,A)TCC(T,A)CA(G,C)CTCAGTTCCTT3'

CL10 : 5'ATGTATATATGTTTGTTGTCTATTTC3'

CL11 : 5'ATGGAAGCCCCAGCTCAGCTTCTCTT3'

CL12A : 5'ATG(A,G)AGT(T,C)(A,T)CAGACCCAGGTCTT(T,C)(A,G)T3'

CL12B : 5'ATGGAGACACATTCTCAGGTCTTTGT3'

CL13 : 5'ATGGATTCACAGGCCCAGGTTCTTAT3'

CL14 : 5'ATGATGAGTCCTGCCCAGTTCCTGTT3'

CL15 : 5'ATGAATTTGCCTGTTCATCTCTTGGTGCT3'

CL16 : 5'ATGGATTTTCAATTGGTCCTCATCTCCTT3'

CL17A : 5'ATGAGGTGCCTA(A,G)CT(C,G)AGTTCCTG(A,G)G3'

CL17B : 5'ATGAAGTACTCTGCTCAGTTTCTAGG3'

CL17C : 5'ATGAGGCATTCTCTTCAATTCTTGGG3'

Each of the above primers has the sequence 5'GGACTGTTCGAAGCCGCCACC3' added to its 5' end.

TABLE 3

Oligonucleotide primers for the 3' ends of mouse Vh and Vl genes.

Light chain (CL12) :

5'GGATACAGTTGGTGCAGCATCCGTACGTTT3'

Heavy chain (R2155) :

5'GCAGATGGGCCCTTCGTTGAGGCTG(A,C)(A,G)GAGAC(G,T,A)GTGA3'

TABLE 4

a) 5' Primer mixtures for light chain PCR reactions

pool 1 : CL2.
pool 2 : CL7.
pool 3 : CL13.
pool 4 : CL6.
pool 5 : CL5A, CL9, CL17A.
pool 6 : CL8.
pool 7 : CL12A.
pool 8 :CL1, CL3, CL4, CL5, CL10, CL11, CL2B, CL14, CL15, CL16, CL17B, CL17C

b) 5' Primer mixtures for heavy chain PCR reactions

pool 1 : CH1, CH2, CH3.CH4.
pool 2 : CH5, CH6, CH7.CH8.
pool 3 : CH9, CH10, CH11.CH12.

Table 5

Primers used in nucleotide sequence analysis

R1053: 5'GCTGACAGACTAACAGACTGTTCC3'
R720: 5'GCTCTCGGAGGTGCTCCT3'

Evaluation of Activities of Chimeric Genes

The activities of the chimeric genes were evaluated by expressing them in mammalian cells and purifying and quantitating the newly synthesised antibodies. The methodology for this is described below, followed by a description of the biochemical and cell based assays used for the biological characterisation of the antibodies.

a) Production of Chimeric hTNF40 Antibody Molecule

Antibody for biological evaluation was produced by transient expression of the appropriate heavy and light chain pairs after co-transfection into Chinese Hamster Ovary (CHO) cells using calcium phosphate precipitation.

On the day prior to transfection, semi-confluent flasks of CHO-L761 cells were trypsinised, the cells counted and T75 flasks set up each with 10^7 cells.

On the next day, the culture medium was changed 3 hours before transfection. For transfection, the calcium phosphate precipitate was prepared by mixing 1.25 ml of 0.25 M $CaCl_2$ containing 50 µg of each of heavy and light chain expression vectors with 1.25 ml of 2 x HBS (16.36 gm NaCl, 11.0 gm HEPES and 0.4 gm Na_2HPO_4 in 1 litre water with the pH adjusted to 7.1 with NaOH) and adding immediately into the medium of the cells. After 3 hours at 37°C in a CO_2 incubator, the medium and precipitate were removed and the cells shocked by the addition of 15 ml 15% glycerol in phosphate buffered saline (PBS) for 1 minute. The glycerol was removed, the cells washed once with PBS and incubated for 48-96 hours in 25 ml medium containing 10 mM sodium butyrate. Antibody is purified from the culture medium by binding to and elution from protein A – Sepharose.

b) ELISA

For the ELISA, Nunc ELISA plates were coated overnight at 4°C with a $F(ab)_2$ fragment of a polyclonal goat anti-human Fc fragment specific antibody (Jackson Immunoresearch, code 109-006-098) at 5 µg/ml in coating buffer (15mM sodium carbonate, 35mM sodium hydrogen carbonate, pH6.9). Uncoated antibody was removed by washing 5 times with distilled water. Samples and purified standards to be quantitated were diluted to approximately 1 µg/ml in conjugate buffer (0.1M Tris-HCl, pH7.0, 0.1M NaCl, 0.2% v/v Tween 20, 0.2% w/v Hammersten casein). The samples were titrated in the microtitre wells in 2-fold dilutions to give a final volume of 0.1 ml in each well and the plates incubated at room temperature for 1 hour with shaking. After the first incubation step the plates were washed 10 times with distilled water and then incubated for 1 hour as before with 0.1 ml of a mouse monoclonal anti-human kappa (clone GD12) peroxidase conjugated antibody (The Binding Site, code MP135) at a dilution of 1 in 700 in conjugate buffer. The plate was washed again and substrate solution (0.1 ml) added to each well. Substrate solution contained 150 µl N,N,N,N-tetramethylbenzidine (10mg/ml in DMSO), 150 µl hydrogen peroxide (30% solution) in 10 ml 0.1M sodium acetate/sodium citrate, pH 6.0. The plate was developed for 5-10 minutes until the absorbance at 630nm was approximately 1.0 for the top standard. Absorbance at 630nm was measured using a plate reader and the concentration of the sample determined by comparing the titration curves with those of the standard.

c) Determination of Affinity constants by BiaCore analysis.

The binding interaction between hTNF40 and human TNF was investigated using BIA technology.

An affinity purified goat polyclonal antibody, directed against the constant region of hTNF40, was immobilised on the dextran polymer sensor chip surface using standard NHS/EDC chemistry. Relatively low levels (200-500 RU) of hTNF40 were captured to ensure mass transport effects were minimised. Human TNF at different concentrations was passed over the captured hTNF40 to allow assessment of the association kinetics. Following the injection of ligand, buffer was passed over the surface so that the dissociation could be measured. The association and dissociation rate constants for the interaction between solid phase hTNF40 and human TNF were calculated, and a K_D value was derived.

EXAMPLE 1

CDR-Grafting of hTNF40

The molecular cloning of genes for the variable regions of the heavy and light chains of the hTNF40 antibody and their use to produce chimeric (mouse-human) hTNF40 antibodies has been described above. The nucleotide and amino acid sequences of the murine hTNF40 Vl and Vh are shown in Figures 6 and 7, respectively. This example describes the CDR-grafting of the hTNF40 antibody.

CDR-Grafting of hTNF40 Light Chain

Alignment of the framework regions of hTNF40 light chain with those of the four human light chain subgroups (Kabat *et al.*, 1991, *supra*) revealed that hTNF40 was most homologous to antibodies in human light chain subgroup 1. Consequently, for constructing the CDR-grafted light chain, the framework regions chosen corresponded to those of the human group 1 consensus sequence.

A comparison of the amino acid sequences of the framework regions of murine hTNF40 and the consensus human group 1 light chains is given in Figure 1 and shows that there are 22 differences (underlined) between the two sequences. Analysis of the contribution that any of these framework differences might have on antigen binding identified 2 residues for investigation; these are at positions 46 and 60. Based on this

analysis, two versions of the CDR-grafted light chain were constructed. In the first of these, hTNF40-gL1, residues 46 and 60 are derived from the hTNF40 light chain while in the second, hTNF40-gL2, all residues are human consensus except residue number 60 is from the nTNF40 light chain.

Construction of CDR-Grafted Light Chain hTNF40-gL1.

The construction of hTNF40-gL1 is given below in detail. The following overlapping oligonucleotides (P7982-P7986) were used in the Polymerase Chain Reactions (PCR) to assemble a truncated grafted light chain. The assembled fragment lacks the antibody leader sequence and the first 17 amino acids of framework 1.

oligo 1 P7982:
5' GAATTCAGGGTCACCATCACTTGTAAAGCCAGTCAGAACGTAGGTACTAAC
GTAGCCTGGTATCAGCAAA3'

oligo 2 P7983:
5' ATAGAGGAAAGAGGCACTGTAGATGAGGGCTTTTGGGGCTTTACCTGGTTT
TTGCTGATACCAGGCTACGT3'

oligo 3 P7984:
5' TACAGTGCCTCTTTCCTCTATAGTGGTGTACCATACAGGTTCAGCGGATCCG
GTAGTGGTACTGATTTCAC3'

oligo 4 P7985
5'GACAGTAATAAGTGGCGAAATCTTCTGGCTGGAGGCTACTGATCGTGAGGGT
GAAATCAGTACCACTACCG3'

oligo 5 P7986:
5'ATTTCGCCACTTATTACTGTCAACAGTATAACATCTACCCACTCACATTCGGT
CAGGGTACTAAAGTAGAAATCAAACGTACGGAATTC3'

Fwd P7981:
5'GAATTCAGGGTCACCATCACTTGTAAAGCC3'

Bwd P7980

5'GAATTCCGTACGTTTGATTTCTACTTTAGT3'

A PCR reaction, 100 µl, was set up containing, 10 mM Tris-HCl pH 8.3., 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 2 pmoles of P7982, P7983, P7984, P7985, P7986 and 10pmoles of P7980, P7981 and 1 unit of Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, each reaction was analysed by electrophoresis on an agarose gel and the PCR fragment excised from the gel and recovered using a Mermaid Kit. The recovered fragment was restricted with the enzymes BstEII and SplI in the appropriate buffer. The resulting product was finally electrophoresed on an agarose gel and the 270 base pair DNA fragment recovered from a gel slice and ligated into vector CTIL5-gL6 (Figure 12), that had previously been digested with the same enzymes. The above vector provides the missing antibody leader sequence and the first 17 amino acids of framework 1.

The ligation mixture was used to transform E. coli strain LM1035 and resulting colonies analysed by PCR, restriction enzyme digests and nucleotide sequencing. The nucleotide and amino acid sequence of the Vl region of hTNF40-gL1 is shown in Figure 8.

Construction of CDR-Grafted Light Chain hTNF40-gL2.

hTNF40-gL2 was constructed using PCR. The following oligonucleotides were used to introduce the amino acid changes:

R1053 : 5'GCTGACAGACTAACAGACTGTTCC3'

R5350:

5'TCTAGATGGCACACCATCTGCTAAGTTTGATGCAGCATAGATCAGGAGCTTAGGAGC3'

R5349:

5'GCAGATGGTGTGCCATCTAGATTCAGTGGCAGTGGATCAGGCACAGACTTTACCCTAAC3'

R684: 5'TTCAACTGCTCATCAGAT3'

Two reactions, each 20 µl, were set up each containing 10 mM Tris-HCl pH 8.3, 1.5mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 0.1 µg hTNF40-gL1, 6 pmoles of R1053/R5350 or R5349/R684 and 0.25 units Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, each reaction was analysed by electrophoresis on an agarose gel and the PCR fragments excised from the gel and recovered using a Mermaid Kit.

Aliquots of these were then subjected to a second round of PCR. The reaction, 100 µl, contained 10 mM Tris-HCl pH 8.3, 1.5mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 1/5 of each of the PCR fragments from the first set of reactions, 30 pmoles of R1053 and R684 and 2.5 units Taq polymerase. Reaction temperatures were as above. After the PCR, the mixture was extracted with phenol/chloroform and then with chloroform and precipitated with ethanol. The ethanol precipitate was recovered by centrifugation, dissolved in the appropriate buffer and restricted with the enzymes BstEII and SplI. The resulting product was finally electrophoresed on an agarose gel and the 270 base pair DNA fragment recovered from a gel slice and ligated into the vector pMR15.1 (Figure 4) that had previously been digested with the same enzymes.

The ligation mixture was used to transform E. coli LM1035 and resulting colonies analysed by PCR, restriction enzyme digests and nucleotide sequencing. The nucleotide and amino acid sequence of the Vl region of hTNF40-glL2 is shown in Figure 9.

CDR-Grafting of hTNF40 Heavy Chain

CDR-grafting of hTNF40 heavy chain was accomplished using the same strategy as described for the light chain. hTNF40 heavy chain was found to be most homologous to human heavy chains belonging to subgroup 1 and therefore the consensus sequence of the human subgroup 1 frameworks was chosen to accept the hTNF40 heavy chain CDRs.

To investigate the requirement of a homologous human framework to act as an acceptor framework for CDR grafting, a second framework, human group 3, was selected to humanise hTNF40 heavy chain.

A comparison of hTNF40 with the two different frameworks region is shown in Figure 2 where it can be seen that hTNF40 differs from the human subgroup 1 consensus at

31 positions (underlined) and differs from the human subgroup 3 consensus at 39 positions (underlined). After analysis of the contribution that any of these might make to antigen binding, residues 27, 68, 70, 72, 73 and 76 were retained in the CDR-grafted heavy chain, gh1hTNF40.4 using the group 1 framework. Residues 27, 68, 70, 72, 73 and 76 were retained in the CDR-grafted heavy chain, gh3hTNF40.4 using the group 3 framework.

Construction of CDR-Grafted Heavy Chain gh1hTNF40.4

gh1hTNF40.4 was assembled by subjecting overlapping oligonucleotides to PCR in the presence of the appropriate primers. The following oligonucleotides were used in the PCR:

Group 1 graft

oligo 1 P7989:
5'GAAGCACCAGGCTTCTTAACCTCTGCTCCTGACTGGACCAGCTGCACCTGAG AGTGCACGAATTC3'

oligo 2 P7990:
5'GGTTAAGAAGCCTGGTGCTTCCGTCAAAGTTTCGTGTAAGGCCTCAGGCTAC GTGTTCACAGACTATGGTA3'

oligo 3 P7991:
5'CCAACCCATCCATTTCAGGCCTTGTCCCGGGGCCTGCTTGACCCAATTCATAC CATAGTCTGTGAACACGT3'

oligo 4 P7995:
5'GGCCTGAAATGGATGGGTTGGATTAATACTTACATTGGAGAGCCTATTTATGT TGACGACTTCAAGGGCAGATTCACGTTC3'

oligo 5 P7992:
5'CCATGTATGCAGTGCGTTGTGGAGGTGTCTAGAGTGAACGTGAATCTGCCCTT GAA3'

oligo 6 P7993:

5'CCACAAGCACTGCATACATGGAGCTGTCATCTCTGAGATCCGAGGACACCGC AGTGTACTAT3'

oligo 7 P7994:

5'GAATTCGGTACCCTGGCCCCAGTAGTCCATGGCATAAGATCTGTATCCTCTAG CACAATAGTACACTGCGGTGTCCTC3'

Fwd: P7988:

5'GAATTCGTGCACTCTCAGGTGCAGCTGGTC3'

Bwd P7987:

5'GAATTCGGTACCCTGGCCCCAGTAGTCCAT3'

The assembly reaction, 100 µl, contained 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 2 pmole of each of p7989, p7990, p7991, p7995, p7992, p7993 and p7994, 10 pmoles of each of p7988 and p7987 and 1 unit Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, the reaction was extracted with phenol/chloroform (1/1), then with chloroform and precipitated with ethanol. After centrifugation, the DNA was dissolved in the appropriate restriction buffer and digested with ApaLI and KpnI. The resulting fragment was isolated from an agarose gel and ligated into pMR14 (Figure 5) that had previously been digested with the same enzymes. pMR14 contains the human gamma 4 heavy chain constant region and so the heavy chain expressed from this vector will be a gamma 4 isotype. The ligation mixture was used to transform *E. coli* LM1035 and resulting bacterial colonies screened by restriction digest and nucleotide sequence analysis. In this way, a plasmid containing the correct sequence for gh1hTNF40.4 was identified (Figure 10).

Construction of CDR-Grafted Heavy Chain gh3hTNF40.4

gh3hTNF40.4 was assembled by subjecting overlapping oligonucleotides to PCR in the presence of the appropriate primers. The following oligonucleotides were used in the PCR:

Group 3 graft

oligo 1 P7999:
5'GATCCGCCAGGCTGCACGAGACCGCCTCCTGACTCGACCAGCTGAACCTCAG
AGTGCACGAATTC3'

oligo 2 P8000:
5'TCTCGTGCAGCCTGGCGGATCGCTGAGATTGTCCTGTGCTGCATCTGGTTACG
TCTTCACAGACTATGGAA3'

oligo 3 P8001
5'CCAACCCATCCATTTCAGGCCCTTTCCCGGGGCCTGCTTAACCCAATTCATTC
CATAGTCTGTGAAGACGT3'

oligo 4 P7995:
5'GGCCTGAAATGGATGGGTTGGATTAATACTTACATTGGAGAGCCTATTTATGT
TGACGACTTCAAGGGCAGATTCACGTTC3'

oligo 5 P7997:
5'GGAGGTATGCTGTTGACTTGGATGTGTCTAGAGAGAACGTGAATCTGCCCTT
GAA3'

oligo 6 P7998:
5'CCAAGTCAACAGCATACCTCCAAATGAATAGCCTGAGAGCAGAGGACACCGC
AGTGTACTAT3'

oligo 7 P7993:
5'GAATTCGGTACCCTGGCCCCAGTAGTCCATGGCATAAGATCTGTATCCTCTAG
CACAATAGTACACTGCGGTGTCCTC3'

Fwd P7996:
5'GAATTCGTGCACTCTGAGGTTCAGCTGGTC3'

Bwd P7987:

5'GAATTCGGTACCCTGGCCCCAGTAGTCCAT3'

The assembly reaction, 100 µl, contained 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 2 pmole of each of p7999, p8000, p8001, p7995, p7997, p7998 and p7993, 10 pmoles of each of p7996 and p7987 and 1 unit Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, the reaction was extracted with phenol/chloroform (1/1), then with chloroform and precipitated with ethanol. After centrifugation, the DNA was dissolved in the appropriate restriction buffer and digested with ApaLI and KpnI. The resulting fragment was isolated from an agarose gel and ligated into pMR14 (Figure 5) that had previously been digested with the same enzymes. pMR14 contained the human gamma 4 heavy chain constant region and so the heavy chain expressed from this vector will be a gamma 4 isotype. The ligation mixture was used to transform *E. coli* LM1035 and resulting bacterial colonies screened by restriction digest and nucleotide sequence analysis. In this way, a plasmid containing the correct sequence for gh3hTNF40.4 was identified (Figure 11).

Production of CDR-Grafted hTNF40 Antibody Molecule.

The CDR-grafted hTNF40 antibody molecule CDP870 is constructed using the *E. coli* vector pTTO-1. The variable regions of antibody hTNF40 are sub-cloned into this vector and the intergenic sequence optimised to create pTTO(CDP870). The pTTO expression vector is designed to give rise to soluble, periplasmic accumulation of recombinant proteins in *E. coli*. The main features of this plasmid are;

(i) Tetracycline resistance marker - antibiotic not inactivated by the product of resistance gene, hence selection for plasmid-containing cells is maintained.

(ii) Low copy number - origin of replication derived from plasmid p15A, which is compatible with plasmids containing colE1 derived replicons.

(iii) Strong, inducible tac promoter for trancription of cloned gene(s).

(iv) $LacI^q$ gene - gives constitutive expression of the lac repressor protein, maintaining the tac promoter in the repressed state until induction with IPTG / allolactose.

(v) OmpA signal sequence - gives periplasmic secretion of cloned gene(s).

(vi) Translational coupling of OmpA signal sequence to a short lacZ peptide, giving efficient initiation of translation.

The vector has been developed for expression of antibody Fab' fragments from a dicistronic message by the design of a method to select empirically the optimum intergenic sequence from a series of four purpose-built cassettes. The application of this in the construction of pTTO(CDP870) is described.

Materials and Methods

DNA techniques

Standard procedures were used for protocols including DNA restriction, agarose gel electrophoresis, ligation and transformation. Restriction enzymes and DNA modifying enzymes were obtained from New England Biolabs or Boehringer Mannheim, and were used according to the supplier's recommendations. DNA fragments were purified from agarose using the GeneClean protocol (BIO 101). Oligonucleotides were supplied by Oswel Oligonucleotide Service and were synthesized at the 40nm scale. Plasmid DNA was isolated using Plasmid DNA Mini / Midi kits from Qiagen. PCR was performed using Perkin Elmer 'Amplitaq' as recommended. DNA sequencing was performed using the Applied Biosystems Taq cycle sequencing kit.

Shake flask induction

E. coli W3110 cultures were grown in L-broth supplemented with tetracycline (7.5μg/ml). For inductions, fresh overnight cultures (grown at 30°C) were diluted to OD_{600} of 0.1 into 200 ml L-broth in a 2L baffled flask and were grown at 30°C in an orbital incubator. At OD_{600} of 0.5, IPTG was added to 200 μM. Samples (normalised for OD) were taken at intervals.

Periplasmic Extraction

Culture samples were chilled on ice (5 minutes) then cells were harvested by centrifugation. Following resuspension in extraction buffer (100mMTris.HCl, 10mM EDTA; pH7.4) samples were incubated overnight at 30°C, then clarified by centrifugation.

Assembly Assay

Fab' concentrations were determined by ELISA. Plates were coated at 4°C overnight with anti-human Fd 6045 (2μg/ml in coating buffer, physiological saline, 100μl per well). After washing, 100μl of sample was loaded per well; purified A5B7 gamma-1 Fab', initially at 2μg/ml, was used as a standard. Samples were serially diluted 2-fold across the plate in sample conjugate buffer (per litre: 6.05g tris aminomethane; 2.92g NaCl;

0.1ml Tween-20; 1ml casein (0.2%)); plates were incubated for 1 hour at room temperature, with agitation. Plates were washed and dried, then 100µl of anti-human C-kappa (GD12)-peroxidase was added (diluted in sample conjugate buffer). Incubation was carried out at room temperature for 1 hour with agitation. Plates were washed and dried, then 100µl of substrate solution was added (10ml sodium acetate/citrate solution (0.1M pH 6); 100µl H_2O_2 solution; 100µl tetramethyl benzidine solution (10mg/ml in dimethylsulphoxide)). Absorbance at 630nm was read 4 - 6 minutes after substrate addition.

Construction of Plasmid pTTO-1

(a) Replacement of the pTTQ9 Polylinker

Plasmid pTTQ9 was obtained from Amersham and is shown in figure 14. An aliquot (2µg) was digested with restriction enzymes SalI and EcoRI, the digest was run on a 1% agarose gel and the large DNA fragment (4520 bp) was purified. Two oligonucleotides were synthesized which, when annealed together, encode the OmpA polylinker region shown in figure 15. This sequence has cohesive ends which are compatible with the SalI and EcoRI ends generated by restriction of pTTQ9. By cloning this oligonucleotide 'cassette' into the pTTQ9 vector, the SalI site is not regenerated, but the EcoRI site is maintained. The cassette encodes the first 13 amino acids of the signal sequence of the *E. coli* outer-membrane protein Omp-A, preceded by the Shine Dalgarno ribosome binding site of the OmpA gene. In addition restriction sites for enzymes XbaI, MunI, StyI and SplI are present. The MunI and StyI sites are within the coding region of the OmpA signal sequence and are intended as the 5' cloning sites for insertion of genes. The two oligonucleotides which make up this cassette were annealed together by mixing at a concentration of 5 pmoles/µl and heating in a waterbath to 95°C for 3 minutes, then slow cooling to room temperature. The annealed sequence was then ligated into the SalI / EcoRI cut pTTQ9. The resulting plasmid intermediate, termed pTQOmp, was verified by DNA sequencing.

(b) Fragment Preparation and Ligation

Plasmid pTTO-1 was constructed by ligating one DNA fragment from plasmid pACYC184 to two fragments generated from pTQOmp. Plasmid pACYC184 was obtained from New England Biolabs, and a restriction map is shown in figure 16. An aliquot (2µg) was digested to completion with restriction enzyme StyI, then treated with Mung Bean Nuclease; this treatment creates blunt ends by cutting back 5' base overhangs. Following phenol extraction and ethanol precipitation, the DNA was restricted with enzyme PvuII, generating fragments of 2348, 1081, 412 and 403 bp. The 2348 bp fragment was purified after agarose gel electrophoresis. This fragment encodes the tetracycline resistance marker

and the p15A origin of replication. The fragment was then treated with calf intestinal alkaline phosphatase to remove 5' terminal phosphates, thereby preventing the self-ligation of this molecule.

An aliquot (2μg) of plasmid pTQOmp was digested with enzymes SspI and EcoRI, and the 2350 bp fragment was purified from unwanted fragments of 2040 bp and 170 bp following agarose gel electrophoresis; this fragment encodes the transcriptional terminator region and the $lacI^q$ gene. Another aliquot (2μg) of pTQOmp was digested with EcoRI and XmnI, generating fragments of 2289, 1670, 350 and 250 bp. The 350 bp fragment, encoding the tac promoter, OmpA signal sequence and multicloning site, was gel purified.

The three fragments were then ligated, using approximately equimolar amounts of each fragment, to generate the plasmid pTTO-1. All cloning junctions were verified by DNA sequencing. The restriction map of this plasmid is shown in figure 17. Plasmid pTTO-2 was then created by insertion of DNA encoding the human Ig light chain kappa constant domain. This was obtained as a Spl I – EcoRI restriction fragment from plasmid pHC132, and inserted into the corresponding sites in pTTO-1. Plasmid pTTO-2 is shown in figure 18.

Insertion of hTNF40 variable regions into pTTO-2

The variable light chain region of hTNF40 was obtained by PCR 'rescue' from the corresponding vector for mammalian cell expression pMR10.1. The OmpA leader sequence replaces the native Ig leader. The sequence of the PCR primers is shown below:

5' primer:
CGCGCGGCAATTGCAGTGGCCTTGGCTGGTTTCGCTACCGTAGCGCAAG
CTGACATTCAAATGACCCAGAGCCC

3' primer: TTCAACTGCTCATCAGATGG

Following PCR under standard conditions, the product was purified, digested with enzymes MunI and SplI then gel purified. The purified fragment was then inserted into the MunI / SplI sites of pTTO-2 to create the light chain intermediate pTTO(hTNF40L).

The variable heavy chain region of hTNF40 was obtained in the same way from the vector pGamma-4. The sequence of the PCR primers is shown below:

5' primer:

GCTATCGCAATTGCAGTGGCGCTAGCTGGTTTCGCCACCGTGGCGCAAG CTGAGGTTCAGCTGGTCGAGTCAGGAGGC

3' primer: GCCTGAGTTCCACGACAC

Following PCR the product was purified, digested with enzymes NheI and ApaI then sub-cloned into the vector pDNAbEng-G1 (figure 19). After verification by DNA sequencing, the heavy chain was restricted with enzyme EcoRI and sub-cloned into the EcoRI site of pTTO(hTNF40L) to create the *E. coli* expression plasmid pTTO(hTNF40).

Optimisation of Intergenic Sequence for Fab' Expression

In the pTTO vector Fab' expression occurs from a dicistronic message encoding first light chain then heavy chain. The DNA sequence between the two genes (intergenic sequence, IGS) can influence the level of expression of the heavy chain by affecting the rate of translational initiation. For example, a short intergenic sequence may result in translational coupling between the light and heavy chains, in that the translating ribosome may not fully dissociate from the mRNA after completing light chain synthesis before initiating heavy chain synthesis. The 'strength' of any Shine Dalgarno (SD) ribosome binding site (homolgy to 16S rRNA) can also have an effect, as can the distance and sequence composition between the SD and the ATG start codon. The potential secondary structure of mRNA around the ATG is another important factor; the ATG should be in a 'loop' and not constrained within a 'stem', while the reverse applies to the SD. Thus by modifying the composition and length of the IGS it is possible to modify the strength of translational initiation and therefore the level of heavy chain production. It is likely that an optimum rate of translational initiation needs to be achieved to maximise expression of the heavy chain of a given Fab'. For example, with one Fab' a high level of expression may be tolerated, but for a different Fab' with different amino acid sequence a high level of expression might prove toxic, perhaps because of different efficiencies of secretion or folding. For this reason a series of four intergenic sequences were designed (figure 20), permitting the empirical determination of the optimum IGS for hTNF40 Fab'. IGS1 and IGS2 have very short intergenic sequences (-1 and +1 respectively) and might be expected to give closely coupled translation; the SD sequences (underlined) are subtly different. These two sequences will most likely confer a high level of translational initiation. IGS3 and IGS4 have a longer distance between start and stop codons (+13) and differ in their sequence composition; IGS3 has a 'stronger' SD sequence. All sequences were studied for secondary structure (using m/fold program) and 'optimised' as far as possible; however,

with tight coupling of translation of the two chains the lack of ribosomal dissociation means that the mRNA may not be 'naked', preventing secondary structure formation.

Cloning of IGS variants

The IGS cassettes shown in figure 20 have flanking SacI and MunI cloning sites. They were built by annealing complementary oligonucleotide pairs. A vector fragment was prepared by digesting pTTO(hTNF40) with SacI and NotI, and a heavy chain fragment was prepared by digesting pDNAbEng G1(hTNF40 H) with MunI and NotI. Three-way ligations were then performed, using equimolar amounts of the two restriction fragments and approximately 0.05 pmoles of each annealed oligo cassette. This created the four expression plasmids pTTO(hTNF40 IGS-1), pTTO(hTNF40 IGS-2), pTTO(hTNF40 IGS-3), pTTO(hTNF40 IGS-4).

Shake flask expression analysis

The four plasmids were transformed into strain W3110, along with the original expression construct, and then analysed for expression in shake flasks as described. The results of a typical experiment are shown in figure 21. The different intergenic sequences confer different expression profiles. IGS1 and IGS2 accumulate periplasmic Fab' rapidly with a peak at 1 hour post induction, after which the level recovered falls. The peak is greater and the fall sharper for IGS1. These results are consistent with a high level of synthesis, as expected for close translational coupling for these constructs. IGS1 apparently confers a higher level of heavy chain expression than does IGS2. In this instance, it appears that this high level of expression is poorly tolerated, since periplasmic expression levels fall after the 1 hour peak. This is seen on the growth profile of the IGS1 culture (not shown), which peaks at 1 hour post induction before falling, suggesting cell death and lysis. IGS3 accumulates Fab' more slowly but peaks at 2 hours post induction with a higher peak value (325 ng/ml/OD), before levels fall. The growth of this culture continued to 3 hours post induction and reached a higher peak biomass (not shown). This is consistent with a lower level of heavy chain synthesis. IGS4 accumulates material at a slower rate still and fails to reach the high peak of productivity of the other 3 constructs. All IGS variants out-perform the original vector significantly. The hypothesis that the different IGS sequences confer different rates of translational initiation is supported by these experimental results. For the hTNF40 Fab' it appears that a high rate of heavy chain translational initiation is poorly tolerated and is therefore not optimal. A slower rate, as conferred by IGS3, results in better growth characteristics and consequently a better yield accumulates over time.

Following comparison of productivity in the fermenter the IGS3 construct was selected as the highest yielding and was termed pTTO(CDP870) – see figure 22.

PEGylation of CDR-Grafted hTNF40 Antibody Molecule.

The purified Fab' is site-specifically conjugated with a molecule of PEG. This is achieved by activation of a single cysteine residue in the hinge region of the Fab', followed by reaction with PEG-maleimide as previously described (A.P. Chapman *et al.* (1999) Nature Biotechnology 17, 780-783). The PEGylated molecule is shown in figure 13.

Efficacy of PEGylated CDR-Grafted hTNF40 Antibody Molecule in Treating Rheumatoid Arthritis.

PEGylated CDR-grafted hTNF40 antibody molecule CDP870 (comprising hTNF40-gL1 and gh3hTNF40.4) has a long half live of approximately 11 days. The antibody molecule was used in a dose-ranging, double-blind, placebo-controlled trial conducted with 36 patients who had active rheumatoid arthritis. The patients were randomised to receive either placebo or CDP870, administered at 1, 5 or 20mg/kg by intravenous infusion. The patients were initially followed for eight weeks following infusion. Their disease activity was assessed by using the standard ACR20 score, defined by the American College of Rheumatology. This employs a range of parameters including the numbers of tender or swollen joints, pain and acute phase reactant levels.

Results

ACR20 score expressed as a percentage of patients who achieved a 20% or greater improvement in disease activity.

	CDP 870 mg/kg			
	Placebo	1	5	20
4wks	16.7	50	87.5	62.5
8wks	16.7	25	75	75

At all dosages a single intravenous infusion of CDP870 produced a rapid and long-lasting improvement, as determined by all disease parameters.

The substantial clinical benefit was maintained throughout the 8 week assessment period by a single treatment with either the 5 or 20 mg/kg dose.

There were significant treatment effects on the number of tender and swollen joints. Doses of 5 mg/kg and 20 mg/kg resulted in reductions of 70-80% in the number of tender joints and 60-7-% reduction in the number of swollen joints present 8 weeks after a single infusion.

CDP 870 was well tolerated in these patients, and its lack of immunogenicity was confirmed.

It should be understood that the above described examples are merely exemplary and do not limit the scope of the present invention as defined in the following claims.

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Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
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<210> 30
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 30
atgaagttgc ctgttaggct gttggtgct 29

<210> 31
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 31
atggagwcag acacactcct gytatgggt 29

<210> 32
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<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 32
atgagtgtgc tcactcaggt cct 23

<210> 33
<211> 26
<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 33
atgaggrccc ctgctcagwt tyttgg 26

<210> 34
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 34
atggatttwc aggtgcagat twtcagctt 29

<210> 35
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 35
atggatttwc argtgcagat twtcagctt 29

<210> 36
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 36
atgaggtkcy ytgytsagyt yctgrg 26

<210> 37
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 37
atgggcwtca agatggagtc aca 23

<210> 38
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 38
atgtggggay ctktttycmm tttttcaat 29

<210> 39
<211> 24
<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 39
atggtrtccw casctcagtt cctt 24

<210> 40
<211> 26
<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 40
atgtatatat gtttgttgtc tatttc 26

<210> 41
<211> 26
<212> DNA
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<220>
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<400> 41
atggaagccc cagctcagct tctctt 26

<210> 42
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
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<400> 42
atgragtywc agacccaggt cttyrt 26

<210> 43
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 43
atggagacac attctcaggt ctttgt 26

<210> 44
<211> 26
<212> DNA
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<220>
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<400> 44
atggattcac aggcccaggt tcttat 26

<210> 45
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 45
atgatgagtc ctgcccagtt cctgtt 26

<210> 46
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 46
atgaatttgc ctgttcatct cttggtgct 29

<210> 47
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
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<400> 47
atggattttc aattggtcct catctccttt 29

<210> 48
<211> 26
<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 48
atgaggtgcc tarctsagtt cctgrg 26

<210> 49
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
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<400> 49
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<210> 50
<211> 26
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<220>
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<400> 50
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<210> 51
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<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 51
ggactgttcg aagccgccac c 21

<210> 52
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 52
ggatacagtt ggtgcagcat ccgtacgttt 30

<210> 53
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
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<400> 53
gcagatgggc ccttcgttga ggctgmrgag acdgtga 37

<210> 54
<211> 24
<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 54
gctgacagac taacagactg ttcc 24

<210> 55
<211> 18
<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 55
gctctcggag gtgctcct 18

<210> 56
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 56
gaattcaggg tcaccatcac ttgtaaagcc agtcagaacg taggtactaa cgtagcctgg 60
tatcagcaaa 70

<210> 57
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 57
atagaggaaa gaggcactgt agatgagggc ttttggggct ttacctggtt tttgctgata 60
ccaggctacg t 71

<210> 58
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 58
tacagtgcct ctttcctgta tagtggtgta ccatacaggt tcagcggatc cggtagtggt 60
actgatttca c 71

<210> 59
<211> 71
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 59
gacagtaata agtggcgaaa tcttctggct ggaggctact gatcgtgagg gtgaaatcag 60
taccactacc g 71

<210> 60
<211> 89
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 60
atttcgccac ttattactgt caacagtata acatctaccc actcacattc ggtcagggta 60
ctaaagtaga aatcaaacgt acggaattc 89

<210> 61
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 61
gaattcaggg tcaccatcac ttgtaaagcc 30

<210> 62
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 62
gaattccgta cgtttgattt ctactttagt 30

<210> 63
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 63
gctgacagac taacagactg ttcc 24

<210> 64
<211> 57
<212> DNA
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<220>
<223> Description of Artificial Sequence:primer

<400> 64
tctagatggc acaccatctg ctaagtttga tgcagcatag atcaggagct taggagc 57

<210> 65
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 65
gcagatggtg tgccatctag attcagtggc agtggatcag gcacagactt taccctaac 59

<210> 66
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 66
ttcaactgct catcagat 18

<210> 67
<211> 65
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 67
gaagcaccag gcttcttaac ctctgctcct gactggacca gctgcacctg agagtgcacg 60
aattc 65

<210> 68
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 68
ggttaagaag cctggtgctt ccgtcaaagt ttcgtgtaag gcctcaggct acgtgttcac 60
agactatggt a 71

<210> 69
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 69
ccaacccatc catttcaggc cttgtcccgg ggcctgcttg acccaattca taccatagtc 60

tgtgaacacg t 71

<210> 70
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 70
ggcctgaaat ggatgggttg gattaatact tacattggag agcctattta tgttgacgac 60
ttcaagggca gattcacgtt c 81

<210> 71
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 71
ccatgtatgc agtgcgttgt ggaggtgtct agagtgaacg tgaatctgcc cttgaa 56

<210> 72
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 72
ccacaagcac tgcatacatg gagctgtcat ctctgagatc cgaggacacc gcagtgtact 60
at 62

<210> 73
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 73
gaattcggta ccctggcccc agtagtccat ggcataagat ctgtatcctc tagcacaata 60
gtacactgcg gtgtcctc 78

<210> 74
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 74
gaattcgtgc actctcaggt gcagctggtc 30

<210> 75

<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 75
gaattcggta ccctggcccc agtagtccat 30

<210> 76
<211> 65
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 76
gatccgccag gctgcacgag accgcctcct gactcgacca gctgaacctc agagtgcacg 60
aattc 65

<210> 77
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 77
tctcgtgcag cctggcggat cgctgagatt gtcctgtgct gcatctggtt acgtcttcac 60
agactatgga a 71

<210> 78
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 78
ccaacccatc catttcaggc cctttcccgg ggcctgctta acccaattca ttccatagtc 60
tgtgaagacg t 71

<210> 79
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 79
ggcctgaaat ggatgggttg gattaatact tacattggag agcctattta tgttgacgac 60
ttcaagggca gattcacgtt c 81

<210> 80
<211> 55
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 80
ggaggtatgc tgttgacttg gatgtgtcta gagagaacgt gaatctgccc ttgaa 55

<210> 81
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 81
ccaagtcaac agcatacctc caaatgaata gcctgagagc agaggacacc gcagtgtact 60
at 62

<210> 82
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 82
gaattcggta ccctggcccc agtagtccat ggcataagat ctgtatcctc tagcacaata 60
gtacactgcg gtgtcctc 78

<210> 83
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 83
gaattcgtgc actctgaggt tcagctggtc 30

<210> 84
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 84
gaattcggta ccctggcccc agtagtccat 30

<210> 85
<211> 74
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 85
cgcgcggcaa ttgcagtggc cttggctggt ttcgctaccg tagcgcaagc tgacattcaa 60
atgacccaga gccc 74

<210> 86
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 86
ttcaactgct catcagatgg 20

<210> 87
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 87
gctatcgcaa ttgcagtggc gctagctggt ttcgccaccg tggcgcaagc tgaggttcag 60
ctggtcgagt caggaggc 78

<210> 88
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 88
gcctgagttc cacgacac 18

<210> 89
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 89
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys
20

<210> 90
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 90
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys
20

<210> 91
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 91
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 92
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 92
Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile Tyr
1 5 10 15

<210> 93
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 93
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
20 25 30

<210> 94
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 94
Gly Val Pro Tyr Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Thr Val Gln Ser Glu Asp Leu Ala Glu Tyr Phe Cys
20 25 30

<210> 95
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 95
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 96
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 96
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
1 5 10

<210> 97
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 97
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

<210> 98
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 98
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr
20 25 30

<210> 99
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 99
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
1 5 10

<210> 100
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 100
Trp Val Lys Gln Ala Pro Gly Lys Ala Phe Lys Trp Met Gly
1 5 10

<210> 101
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artifical sequence

<400> 101
Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 102
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 102
Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe Leu Gln
1 5 10 15

Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg
20 25 30

<210> 103
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 103
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 104
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 104
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

<210> 105
<211> 324
<212> DNA
<213> murine

<220>
<221> CDS
<222> (1)..(324)

<400> 105

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gac att gtg atg acc cag tct caa aaa ttc atg tcc aca tca gta gga   48
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
 1               5                  10                  15

gac agg gtc agc gtc acc tgc aag gcc agt cag aat gtg ggt act aat   96
Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
            20                  25                  30

gta gcc tgg tat caa cag aaa cca gga caa tct cct aaa gca ctg att   144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
        35                  40                  45

tac tcg gca tcc ttc cta tat agt gga gtc cct tat cgc ttc aca ggc   192
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Tyr Arg Phe Thr Gly
    50                  55                  60

agt gga tct ggg aca gat ttc act ctc acc atc agc act gtg cag tct   240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Thr Val Gln Ser
65                  70                  75                  80

gaa gac ttg gca gag tat ttc tgt cag caa tat aac atc tat cct ctc   288
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ile Tyr Pro Leu
                85                  90                  95

acg ttc ggt gct ggg acc aag ctg gag ctg aaa cgt                   324
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
            100                 105
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<210> 106
<211> 108
<212> PRT
<213> murine

<400> 106

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Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
 1               5                  10                  15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Tyr Arg Phe Thr Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Thr Val Gln Ser
65                  70                  75                  80

Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ile Tyr Pro Leu
                85                  90                  95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
            100                 105
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<210> 107
<211> 354
<212> DNA
<213> murine

<220>
<221> CDS
<222> (1)..(354)

<400> 107

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cag atc cag ttg gtg cag tct gga cct gag ctg aag aag cct gga gag   48
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1               5                  10                  15

aca gtc aag atc tcc tgc aag gct tct gga tat gtt ttc aca gac tat   96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

gga atg aat tgg gtg aag cag gct cca gga aag gct ttc aag tgg atg   144
Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Ala Phe Lys Trp Met
        35                  40                  45

ggc tgg ata aac acc tac att gga gag cca ata tat gtt gat gac ttc   192
Gly Trp Ile Asn Thr Tyr Ile Gly Glu Pro Ile Tyr Val Asp Asp Phe
    50                  55                  60

aag gga cga ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc ttt   240
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
65                  70                  75                  80

ttg cag atc aac aac ctc aaa aat gag gac acg gct aca tat ttc tgt   288
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
                85                  90                  95

gca aga ggt tac cgg tcc tat gct atg gac tac tgg ggt caa gga acc   336
Ala Arg Gly Tyr Arg Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

tca gtc acc gtc tct tca                                          354
Ser Val Thr Val Ser Ser
        115
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<210> 108
<211> 118
<212> PRT
<213> murine

<400> 108

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Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1               5                  10                  15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Ala Phe Lys Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Ile Gly Glu Pro Ile Tyr Val Asp Asp Phe
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50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Arg Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
115

<210> 109
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (29)..(67)

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 109
tcgagttcta gataacgagg cgtaaaaa atg aaa aag aca gct atc gca att 52
Met Lys Lys Thr Ala Ile Ala Ile
1 5

gca gtg gcc ttg gct ctgacgtacg agtcagg 84
Ala Val Ala Leu Ala
10

<210> 110
<211> 13
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

<400> 110
Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala
1 5 10

<210> 111
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (2)..(40)

<220>
<221> CDS

<222> (43)..(66)

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 111
g agc tca cca gta aca aaa agt ttt aat aga gga gag tgt ta atg aag 48
Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Xaa Xaa Lys
1 5 10 15

aag act gct ata gca att g 67
Lys Thr Ala Ile Ala Ile
20

<210> 112
<211> 13
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

<400> 112
Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Xaa
1 5 10

<210> 113
<211> 8
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

<400> 113
Xaa Lys Lys Thr Ala Ile Ala Ile
1 5

<210> 114
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (2)..(43)

<220>
<221> CDS
<222> (45)..(68)

<220>
<223> Description of Artificial Sequence:artificial sequence

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1 5 10 15

aag aag act gct ata gca att g 69
Lys Lys Thr Ala Ile Ala Ile
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<210> 115
<211> 14
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

<400> 115
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1 5 10

<210> 116
<211> 8
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

<400> 116
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1 5

<210> 117
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (2)..(43)

<220>
<221> CDS
<222> (57)..(80)

<220>
<223> Description of Artificial Sequence:artificial sequence

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Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
1 5 10

ggaggaaaaa aaa atg aag aaa act gct ata gca att g 81
Met Lys Lys Thr Ala Ile Ala Ile
15 20

<210> 118
<211> 14
<212> PRT
<213> Artificial Sequence

<223> Description of Artificial Sequence:artificial sequence

<400> 118
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1 5 10

<210> 119
<211> 8
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

<400> 119
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1 5

<210> 120
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<212> DNA
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<222> (2)..(43)

<220>
<221> CDS
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<220>
<223> Description of Artificial Sequence:artificial sequence

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Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
1 5 10

cgaggattat ata atg aag aaa act gct ata gca att g 81
Met Lys Lys Thr Ala Ile Ala Ile
15 20

<210> 121
<211> 14
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

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1 5 10

<210> 122
<211> 8

<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:artificial sequence

<400> 122
Met Lys Lys Thr Ala Ile Ala Ile
1 5

<210> 123
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

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1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> 124
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 124
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr
20 25 30

<210> 125
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<220>
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<210> 126
<211> 14
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<213> Artificial Sequence

<220>
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<210> 127
<211> 32
<212> PRT
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<220>
<223> Description of Artificial Sequence:artificial sequence

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1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 128
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

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20 25 30

<210> 129
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 129

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1 5 10

<210> 130
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:artificial sequence

<400> 130
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

Claims

1. An antibody molecule having specificity for human TNFα, comprising a heavy chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO: 1 for CDR1, SEQ ID NO: 2 for CDR2 or SEQ ID NO: 3 for CDR3.

2. An antibody molecule having specificity for human TNFα, comprising a light chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO: 4 for CDR1, SEQ ID NO: 5 for CDR2 or SEQ ID NO: 6 for CDR3.

3. An antibody molecule having specificity for human TNFα, comprising a heavy chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO:1 for CDR1, SEQ ID NO:2 for CDR2 or SEQ ID NO:3 for CDR3 and a light chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO:4 for CDR1, SEQ ID NO:5 for CDR2 or SEQ ID NO:6 for CDR3.

4. An antibody molecule according to any one of the preceding claims, which is a CDR-grafted antibody molecule.

5. The antibody molecule according to claim 4, wherein the variable domain comprises a human acceptor framework region and non-human donor CDRs.

6. An antibody molecule according to claim 4 or claim 5, wherein the human acceptor framework of the variable domain of the heavy chain comprises non-human donor residues at positions 27, 68, 70, 72, 73 and 76.

7. An antibody molecule according to any one of claims 4 to 6, wherein the human acceptor framework of the variable domain of the light chain comprises non-human donor residues at positions 46 and 60.

8. The antibody molecule according to any one of the preceding claims, comprising the light chain variable region hTNF40-gL1 and the heavy chain variable domain gh3hTNF40.4.

9. A variant of the antibody molecule according to any one of the preceding claims, which has an improved affinity for TNFα.

10. The variant of claim 9 which is obtained by an affinity maturation protocol.

11. The antibody molecule according to any one of the preceding claims which has been PEGylated.

12. A DNA sequence which encodes the antibody molecule according to any one of the preceding claims or the heavy and/or light chain of the antibody molecule.

13. A DNA sequence according to claim 12 comprising the sequence shown in SEQ ID NO: 8 or 10.

14. A DNA sequence according to claim 12 comprising the sequence shown in SEQ ID NO: 12 or 14.

15. A cloning or expression vector containing a DNA sequence according to any one of claims 12 to 14.

16. An *E. coli* expression vector comprising the DNA sequence according to any one of claims 12 to 14.

17. The *E. coli* expression vector according to claim 16 which is pTTO.

18. A host cell transformed with the vector according to any one of claims 15 to 17.

19. A process for the production of an antibody molecule according to any one of claims 1 to 11, comprising culturing the host cell of claim 18 and isolating the antibody molecule.

20. A process for the production of an antibody molecule according to any one of claims 1 to 11, comprising culturing *E. coli* comprising an *E. coli* expression vector comprising the DNA sequence of any one of claims 12 to 14 and isolating the antibody molecule.

21. The process according to claim 20 wherein the antibody molecule is targeted to the periplasm.

22. A therapeutic or diagnostic composition comprising an antibody molecule according to any one of claims 1 to 11.

23. An antibody molecule according to any one of claims 1 to 11 for use in treating a pathology mediated by TNFα.

24. The antibody molecule of claim 23, for use in treating rheumatoid- or osteo-arthritis.

25. Use of the antibody molecule according to any one of claims 1 to 11 in the manufacture of a medicament for the treatment of a pathology mediated by TNFα.

26. The use according to claim 25, wherein the pathology is rheumatoid- or osteo-arthritis.

27. The murine anti-TNF monoclonal antibody hTNF40.

28. A chimeric antibody molecule comprising the light and heavy chain variable domains of the monoclonal antibody of claim 27.

Light chain Comparisons

```
Hu group 1 consensus :  DIQMTQSPSSLSASVGDRVTITC
hTNF40               :  DIVMTQSQKFMSTSVGDRVSVTC

Hu Group 1 consensus :  WYQQKPGKAPKLLIY
hTNF40               :  WYQQKPGQSPKALIY

Hu Group 1 consensus :  GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
hTNF40               :  GVPYRFTGSGSGTDFTLTISTVQSEDLAEYFC

Hu Group 1 consensus :  FGQGTKVEIKR
hTNF40               :  FGAGTKLELKR
```

Figure 1

Figure 2

Heavy chain Group 1 comparisons.

```
Hu Group 1 consensus    : QVQLVQSGAEVKKPGASVKVSCKASGYTFT
hTNF40                  : QIQLVQSGPELKKPGETVKISCKASGYVFT

Hu Group 1 consensus    : WVRQAPGQGLEWMG
hTNF40                  : WVKQAPGKAFKWMG

Hu Group 1 consensus    :RVTITRDTSTSTAYMELSSLRSEDTAVYYCAR
hTNF40                  :RFAFSLETSASTAFLQINNLKNEDTATYFCAR

Hu Group 1 consensus    : WGQGTLVTVSS
hTNF40                  : WGQGTTLTVSS
```

Heavy chain Group 3 comparisons.

```
Hu Group 3 consensus    : EVQLVESGGGLVQPGGSLRLSCAASGFTFS
hTNF40                  : QIQLVQSGPELKKPGETVKISCKASGYVFT

Hu Group 3 consensus    : WVRQAPGKGLEWVS
hTNF40                  : WVKQAPGKAFKWMG

Hu Group 3 consensus    :RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
hTNF40                  :RFAFSLETSASTAFLQINNLKNEDTATYFCAR

Hu Group 3 consensus    : WGQGTLVTVSS
hTNF40                  : WGQGTTLTVSS
```

Figure 3

Sequence of CDRs of hTNF40

H1 DYGMN (SEQ ID NO:1)

H2 WINTYIGEPIYVDDFKG (SEQ ID NO:7)

H2'WINTYIGEPIYADSVKG (SEQ ID NO:2)

H3 GYRSYAMDY (SEQ ID NO:3)

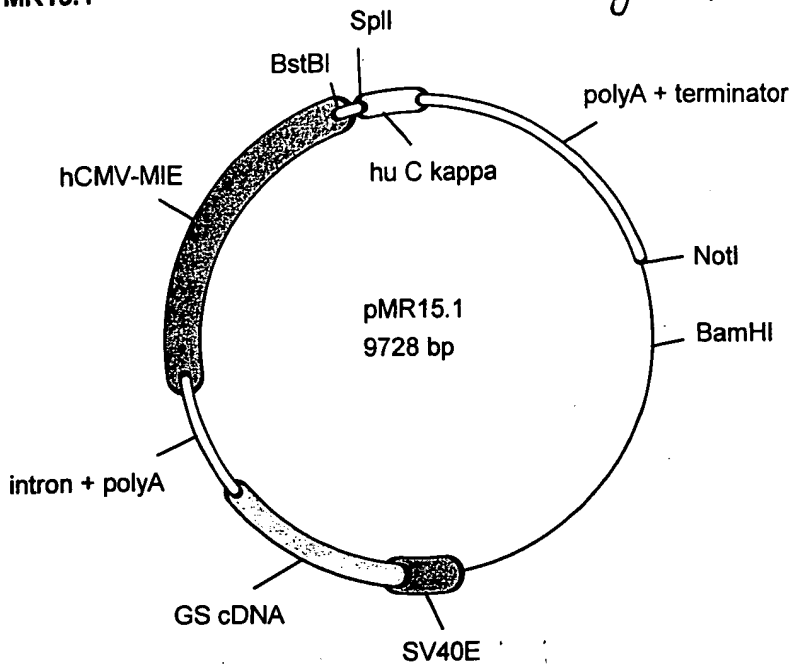
L1 KASQNVGTNVA (SEQ ID NO:4)

L2 SASFLYS (SEQ ID NO:5)

L3 QQYNIYPLT (SEQ ID NO:6)

Plasmid PMR15.1

Figure 4



Plasmid PMR14

Figure 5

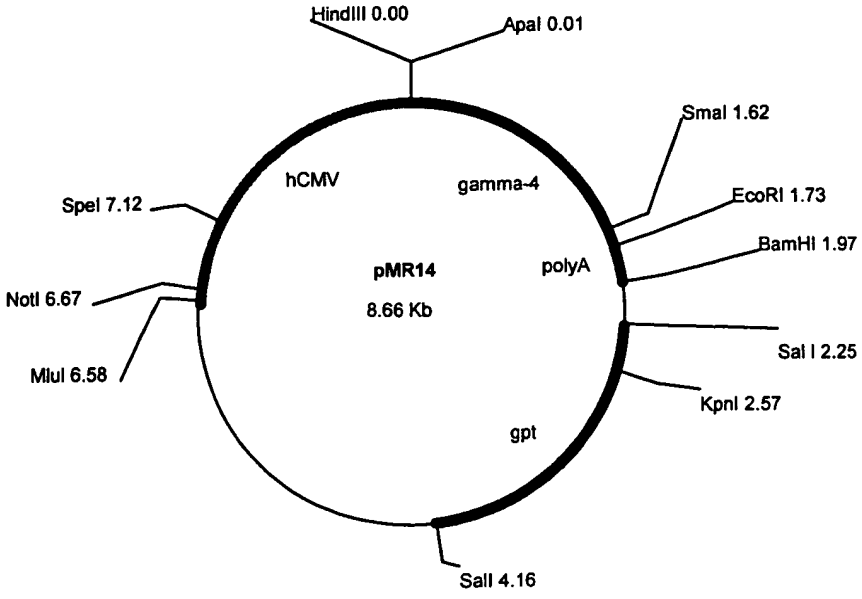


FIG. 6

Murine Vl Sequence of hTNF40

```
          10          20          30          40          50
GAC ATT GTG ATG ACC CAG TCT CAA AAA TTC ATG TCC ACA TCA GTA GGA GAC AGG
CTG TAA CAC TAC TGG GTC AGA GTT TTT AAG TAC AGG TGT AGT CAT CCT CTG TCC
 D   I   V   M   T   Q   S   Q   K   F   M   S   T   S   V   G   D   R>

     60          70          80          90          100
GTC AGC GTC ACC TGC AAG GCC AGT CAG AAT GTG GGT ACT AAT GTA GCC TGG TAT
CAG TCG CAG TGG ACG TTC CGG TCA GTC TTA CAC CCA TGA TTA CAT CGG ACC ATA
 V   S   V   T   C   K   A   S   Q   N   V   G   T   N   V   A   W   Y>

110         120         130         140         150         160
CAA CAG AAA CCA GGA CAA TCT CCT AAA GCA CTG ATT TAC TCG GCA TCC TTC CTA
GTT GTC TTT GGT CCT GTT AGA GGA TTT CGT GAC TAA ATG AGC CGT AGG AAG GAT
 Q   Q   K   P   G   Q   S   P   K   A   L   I   Y   S   A   S   F   L>

       170         180         190         200         210
TAT AGT GGA GTC CCT TAT CGC TTC ACA GGC AGT GGA TCT GGG ACA GAT TTC ACT
ATA TCA CCT CAG GGA ATA GCG AAG TGT CCG TCA CCT AGA CCC TGT CTA AAG TGA
 Y   S   G   V   P   Y   R   F   T   G   S   G   S   G   T   D   F   T>

  220         230         240         250         260         270
CTC ACC ATC AGC ACT GTG CAG TCT GAA GAC TTG GCA GAG TAT TTC TGT CAG CAA
GAG TGG TAG TCG TGA CAC GTC AGA CTT CTG AAC CGT CTC ATA AAG ACA GTC GTT
 L   T   I   S   T   V   Q   S   E   D   L   A   E   Y   F   C   Q   Q>

          280         290         300         310         320
TAT AAC ATC TAT CCT CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGT
ATA TTG TAG ATA GGA GAG TGC AAG CCA CGA CCC TGG TTC GAC CTC GAC TTT GCA
 Y   N   I   Y   P   L   T   F   G   A   G   T   K   L   E   L   K   R>
```

FIG. 7

Murine Vh Sequence of hTNF40

```
          10          20          30          40          50
CAG ATC CAG TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA GAG ACA GTC
GTC TAG GTC AAC CAC GTC AGA CCT GGA CTC GAC TTC TTC GGA CCT CTC TGT CAG
 Q   I   Q   L   V   Q   S   G   P   E   L   K   K   P   G   E   T   V>

     60          70          80          90          100
AAG ATC TCC TGC AAG GCT TCT GGA TAT GTT TTC ACA GAC TAT GGA ATG AAT TGG
TTC TAG AGG ACG TTC CGA AGA CCT ATA CAA AAG TGT CTG ATA CCT TAC TTA ACC
 K   I   S   C   K   A   S   G   Y   V   F   T   D   Y   G   M   N   W>

110         120         130         140         150         160
GTG AAG CAG GCT CCA GGA AAG GCT TTC AAG TGG ATG GGC TGG ATA AAC ACC TAC
CAC TTC GTC CGA GGT CCT TTC CGA AAG TTC ACC TAC CCG ACC TAT TTG TGG ATG
 V   K   Q   A   P   G   K   A   F   K   W   M   G   W   I   N   T   Y>

       170         180         190         200         210
ATT GGA GAG CCA ATA TAT GTT GAT GAC TTC AAG GGA CGA TTT GCC TTC TCT TTG
TAA CCT CTC GGT TAT ATA CAA CTA CTG AAG TTC CCT GCT AAA CGG AAG AGA AAC
 I   G   E   P   I   Y   V   D   D   F   K   G   R   F   A   F   S   L>

  220         230         240         250         260         270
GAA ACC TCT GCC AGC ACT GCC TTT TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC
CTT TGG AGA CGG TCG TGA CGG AAA AAC GTC TAG TTG TTG GAG TTT TTA CTC CTG
 E   T   S   A   S   T   A   F   L   Q   I   N   N   L   K   N   E   D>

          280         290         300         310         320
ACG GCT ACA TAT TTC TGT GCA AGA GGT TAC CGG TCC TAT GCT ATG GAC TAC TGG
TGC CGA TGT ATA AAG ACA CGT TCT CCA ATG GCC AGG ATA CGA TAC CTG ATG ACC
 T   A   T   Y   F   C   A   R   G   Y   R   S   Y   A   M   D   Y   W>

     330         340         350
GGT CAA GGA ACC TCA GTC ACC GTC TCT TCA
CCA GTT CCT TGG AGT CAG TGG CAG AGA AGT
 G   Q   G   T   S   V   T   V   S   S>
```

FIG. 8

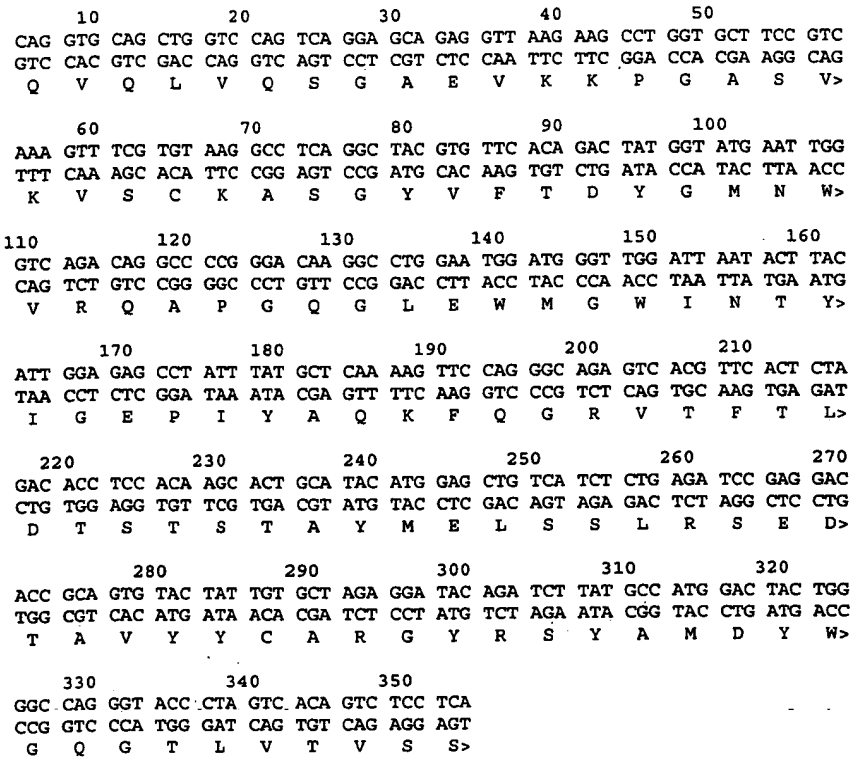
Grafted Vl Sequence of hTNF40

```
          10          20          30          40          50
GAC ATT CAA ATG ACC CAG AGC CCA TCC AGC CTG AGC GCA TCT GTA GGA GAC CGG
CTG TAA GTT TAC TGG GTC TCG GGT AGG TCG GAC TCG CGT AGA CAT CCT CTG GCC
 D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R>

     60          70          80          90          100
GTC ACC ATC ACT TGT AAA GCC AGT CAG AAC GTA GGT ACT AAC GTA GCC TGG TAT
CAG TGG TAG TGA ACA TTT CGG TCA GTC TTG CAT CCA TGA TTG CAT CGG ACC ATA
 V   T   I   T   C   K   A   S   Q   N   V   G   T   N   V   A   W   Y>

110         120         130         140         150         160
CAG CAA AAA CCA GGT AAA GCC CCA AAA GCC CTC ATC TAC AGT GCC TCT TTC CTC
GTC GTT TTT GGT CCA TTT CGG GGT TTT CGG GAG TAG ATG TCA CGG AGA AAG GAG
 Q   Q   K   P   G   K   A   P   K   A   L   I   Y   S   A   S   F   L>

       170         180         190         200         210
TAT AGT GGT GTA CCA TAC AGG TTC AGC GGA TCC GGT AGT GGT ACT GAT TTC ACC
ATA TCA CCA CAT GGT ATG TCC AAG TCG CCT AGG CCA TCA CCA TGA CTA AAG TGG
 Y   S   G   V   P   Y   R   F   S   G   S   G   S   G   T   D   F   T>

  220         230         240         250         260         270
CTC ACG ATC AGT AGC CTC CAG CCA GAA GAT TTC GCC ACT TAT TAC TGT CAA CAG
GAG TGC TAG TCA TCG GAG GTC GGT CTT CTA AAG CGG TGA ATA ATG ACA GTT GTC
 L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q>

          280         290         300         310         320
TAT AAC ATC TAC CCA CTC ACA TTC GGT CAG GGT ACT AAA GTA GAA ATC AAA
ATA TTG TAG ATG GGT GAG TGT AAG CCA GTC CCA TGA TTT CAT CTT TAG TTT
 Y   N   I   Y   P   L   T   F   G   Q   G   T   K   V   E   I   K>
```

FIG. 9

```
          10          20          30          40          50
GAC ATT CAA ATG ACC CAG AGC CCA TCC AGC CTG AGC GCA TCT GTA GGA GAC CGG
CTG TAA GTT TAC TGG GTC TCG GGT AGG TCG GAC TCG CGT AGA CAT CCT CTG GCC
 D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R>

     60          70          80          90          100
GTC ACC ATC ACT TGT AAA GCC AGT CAG AAC GTA GGT ACT AAC GTA GCC TGG TAT
CAG TGG TAG TGA ACA TTT CGG TCA GTC TTG CAT CCA TGA TTG CAT CGG ACC ATA
 V   T   I   T   C   K   A   S   Q   N   V   G   T   N   V   A   W   Y>

110         120         130         140         150         160
CAG CAA AAA CCA GGT AAA GCC CCA AAA CTC CTC ATC TAC AGT GCC TCT TTC CTC
GTC GTT TTT GGT CCA TTT CGG GGT TTT GAG GAG TAG ATG TCA CGG AGA AAG GAG
 Q   Q   K   P   G   K   A   P   K   L   L   I   Y   S   A   S   F   L>

       170         180         190         200         210
TAT AGT GGT GTA CCA TAC AGG TTC AGC GGA TCC GGT AGT GGT ACT GAT TTC ACC
ATA TCA CCA CAT GGT ATG TCC AAG TCG CCT AGG CCA TCA CCA TGA CTA AAG TGG
 Y   S   G   V   P   Y   R   F   S   G   S   G   S   G   T   D   F   T>

  220         230         240         250         260         270
CTC ACG ATC AGT AGC CTC CAG CCA GAA GAT TTC GCC ACT TAT TAC TGT CAA CAG
GAG TGC TAG TCA TCG GAG GTC GGT CTT CTA AAG CGG TGA ATA ATG ACA GTT GTC
 L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q>

          280         290         300         310         320
TAT AAC ATC TAC CCA CTC ACA TTC GGT CAG GGT ACT AAA GTA GAA ATC AAA
ATA TTG TAG ATG GGT GAG TGT AAG CCA GTC CCA TGA TTT CAT CTT TAG TTT
 Y   N   I   Y   P   L   T   F   G   Q   G   T   K   V   E   I   K>
```

FIG. 10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V R Q A P G Q G L E W M G W I N T Y>
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CTG TGG AGG TGT TCG TGA CGT ATG TAC CTC GAC AGT AGA GAC TCT AGG CTC CTG
D T S T S T A Y M E L S S L R S E D>
280 290 300 310 320
ACC GCA GTG TAC TAT TGT GCT AGA GGA TAC AGA TCT TAT GCC ATG GAC TAC TGG
TGG CGT CAC ATG ATA ACA CGA TCT CCT ATG TCT AGA ATA CGG TAC CTG ATG ACC
T A V Y Y C A R G Y R S Y A M D Y W>
330 340 350
GGC CAG GGT ACC CTA GTC ACA GTC TCC TCA
CCG GTC CCA TGG GAT CAG TGT CAG AGG AGT
G Q G T L V T V S S>

FIG. 11

Grafted Vh Sequence of hTNF40.4

```
          10          20          30          40          50
GAG GTT CAG CTG GTC GAG TCA GGA GGC GGT CTC GTG CAG CCT GGC GGA TCA CTG
CTC CAA GTC GAC CAG CTC AGT CCT CCG CCA GAG CAC GTC GGA CCG CCT AGT GAC
 E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   G   S   L>

     60          70          80          90          100
AGA TTG TCC TGT GCT GCA TCT GGT TAC GTC TTC ACA GAC TAT GGA ATG AAT TGG
TCT AAC AGG ACA CGA CGT AGA CCA ATG CAG AAG TGT CTG ATA CCT TAC TTA ACC
 R   L   S   C   A   A   S   G   Y   V   F   T   D   Y   G   M   N   W>

110         120         130         140         150         160
GTT AGA CAG GCC CCG GGA AAG GGC CTG GAA TGG ATG GGT TGG ATT AAT ACT TAC
CAA TCT GTC CGG GGC CCT TTC CCG GAC CTT ACC TAC CCA ACC TAA TTA TGA ATG
 V   R   Q   A   P   G   K   G   L   E   W   M   G   W   I   N   T   Y>

       170         180         190         200         210
ATT GGA GAG CCT ATT TAT GCT GAC AGC GTC AAG GGC AGA TTC ACG TTC TCT CTA
TAA CCT CTC GGA TAA ATA CGA CTG TCG CAG TTC CCG TCT AAG TGC AAG AGA GAT
 I   G   E   P   I   Y   A   D   S   V   K   G   R   F   T   F   S   L>

  220         230         240         250         260         270
GAC ACA TCC AAG TCA ACA GCA TAC CTC CAA ATG AAT AGC CTG AGA GCA GAG GAC
CTG TGT AGG TTC AGT TGT CGT ATG GAG GTT TAC TTA TCG GAC TCT CGT CTC CTG
 D   T   S   K   S   T   A   Y   L   Q   M   N   S   L   R   A   E   D>

          280         290         300         310         320
ACC GCA GTG TAC TAT TGT GCT AGA GGA TAC AGA TCT TAT GCC ATG GAC TAC TGG
TGG CGT CAC ATG ATA ACA CGA TCT CCT ATG TCT AGA ATA CGG TAC CTG ATG ACC
 T   A   V   Y   Y   C   A   R   G   Y   R   S   Y   A   M   D   Y   W>

    330         340         350
GGC CAG GGT ACC CTA GTC ACA GTC TCC TCA
CCG GTC CCA TGG GAT CAG TGT CAG AGG AGT
 G   Q   G   T   L   V   T   V   S   S>
```

FIG. 12

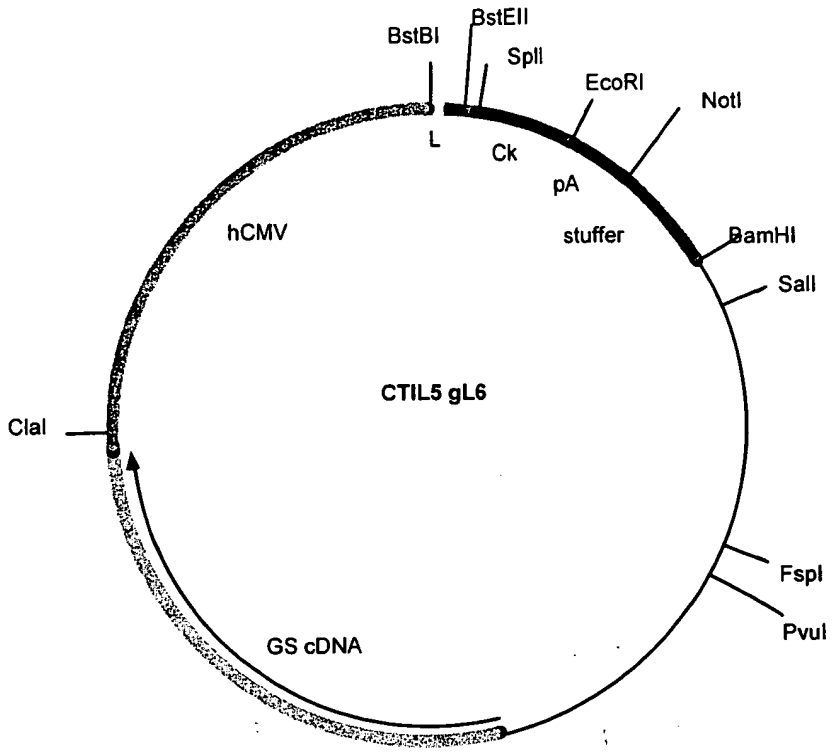

BstBI
BstEII
SpII
EcoRI
NotI
L
Ck
pA
hCMV
stuffer
BamHI
SalI
CTIL5 gL6
ClaI
FspI
PvuI
GS cDNA

Figure 13

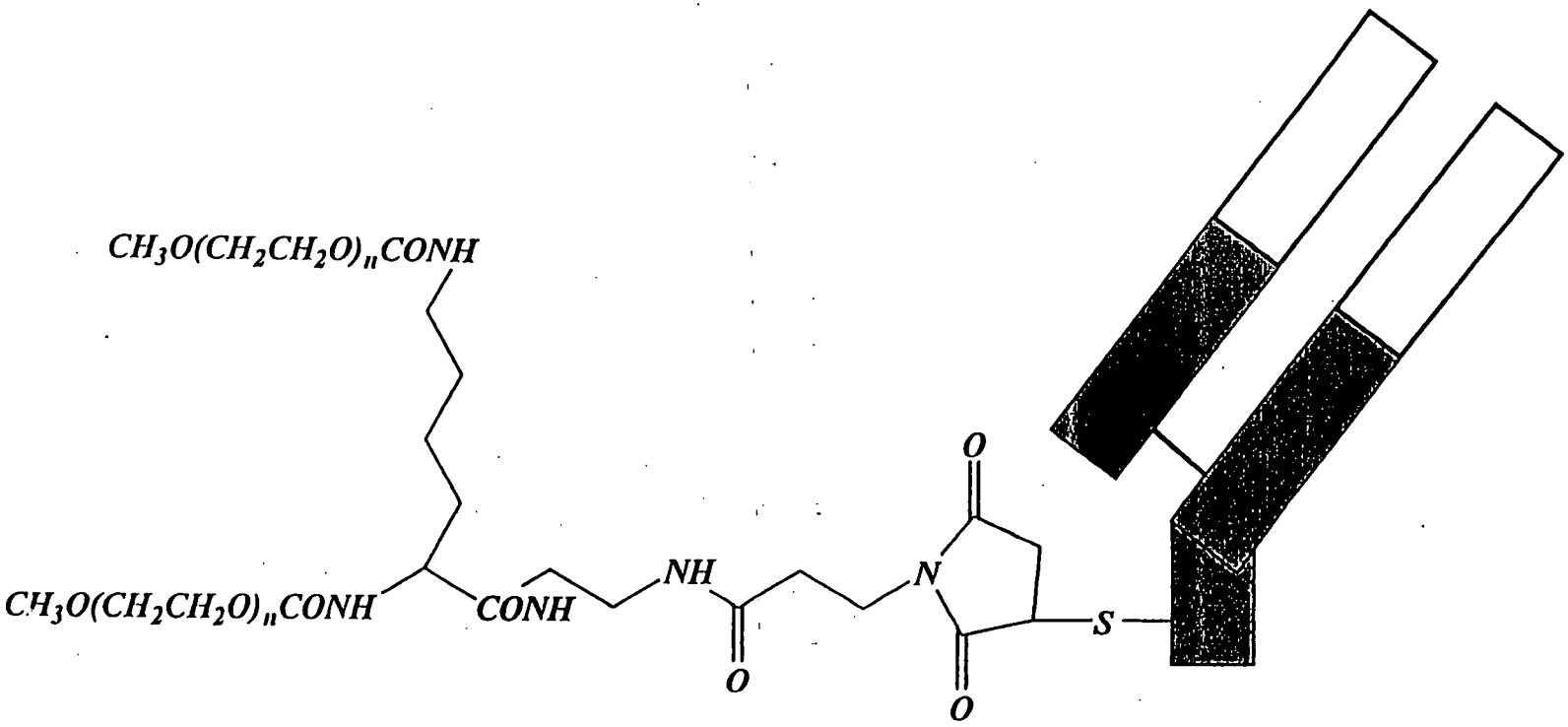


Figure 14

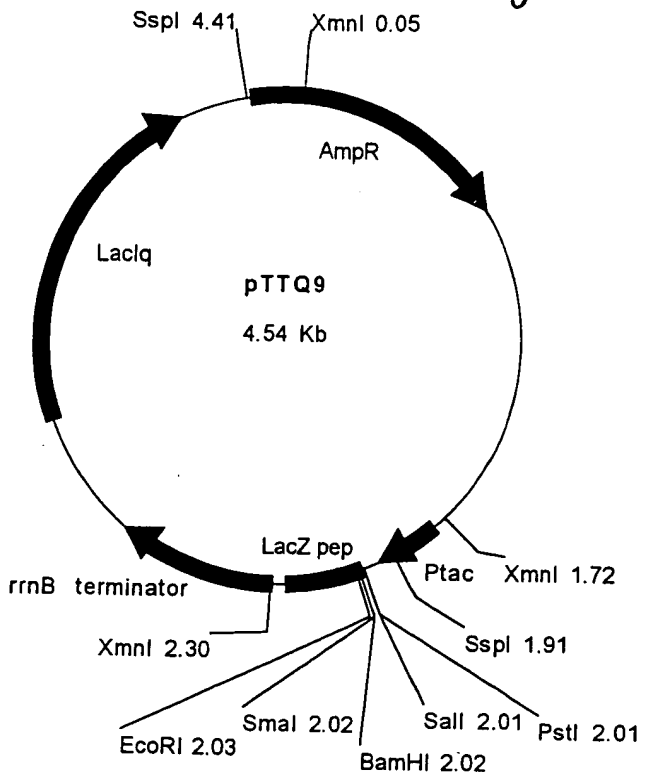
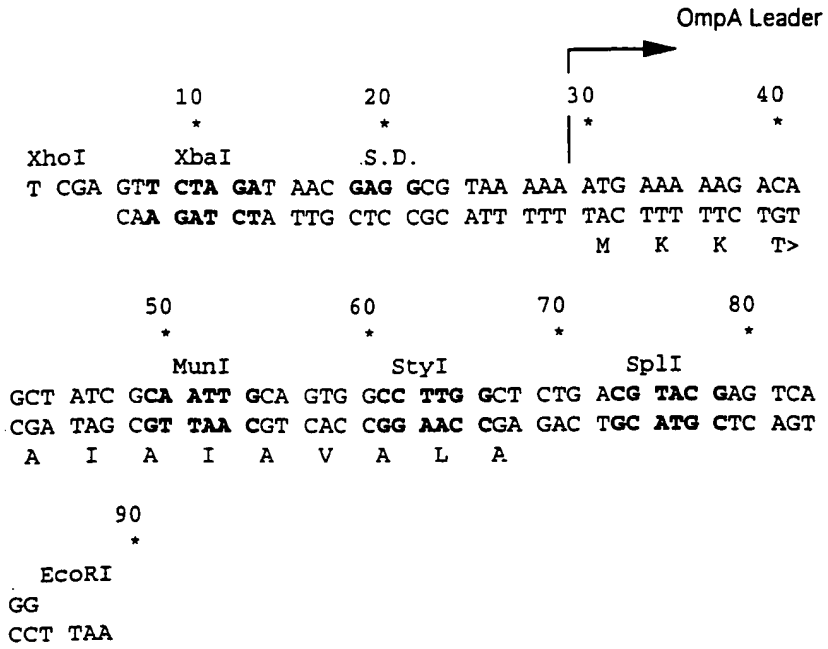

SspI 4.41
XmnI 0.05
AmpR
LacIq
pTTQ9
4.54 Kb
LacZ pep
Ptac
XmnI 1.72
rrnB terminator
XmnI 2.30
SspI 1.91
EcoRI 2.03
SmaI 2.02
SalI 2.01
PstI 2.01
BamHI 2.02

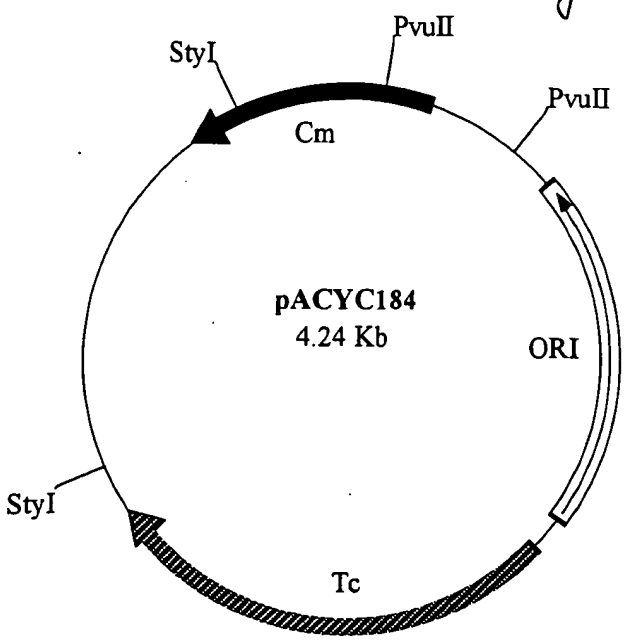
Figure 15

Sequence of OmpA Oligonucleotide Adapter



- Internal restriction sites are shown in bold
- The 5' XhoI cohesive end ligates into the vectorSalI site, blocking it
- S.D. represents the OmpA Shine Dalgarno sequence

Figure 16



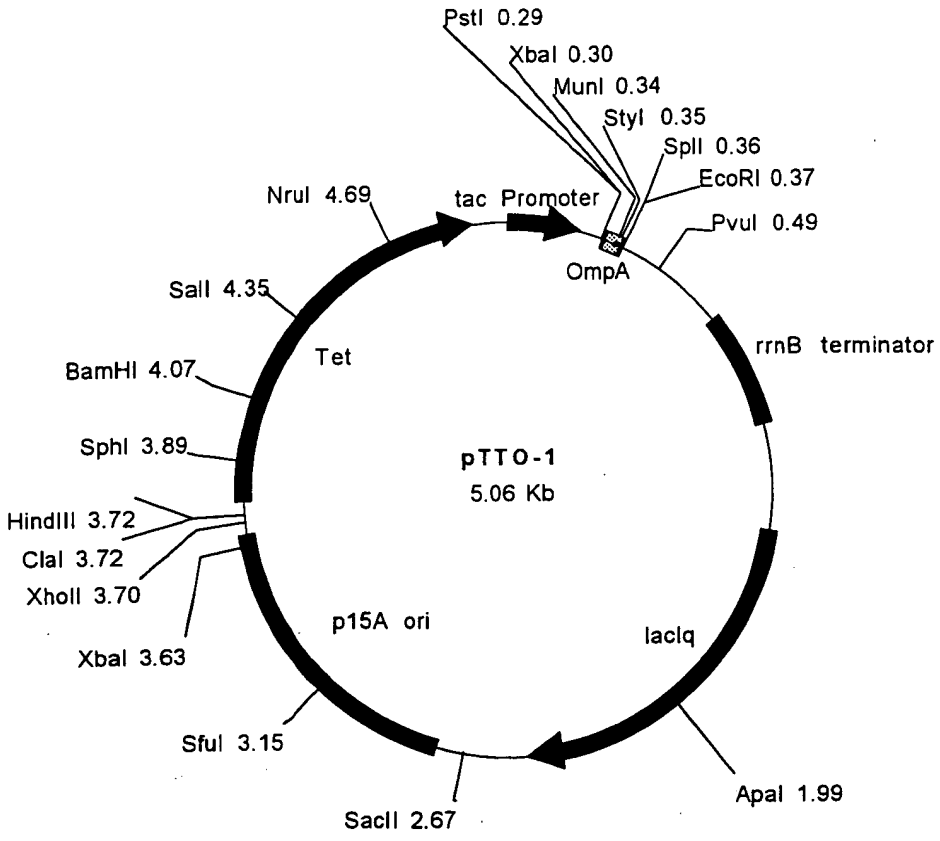

PstI 0.29
XbaI 0.30
MunI 0.34
StyI 0.35
SpII 0.36
EcoRI 0.37
Pvul 0.49
OmpA
rrnB terminator
lacIq
ApaI 1.99
SacII 2.67
Sful 3.15
p15A ori
Xbal 3.63
XhoII 3.70
ClaI 3.72
HindIII 3.72
SphI 3.89
BamHI 4.07
SalI 4.35
Tet
NruI 4.69
tac Promoter
pTTO-1
5.06 Kb

Figure 17

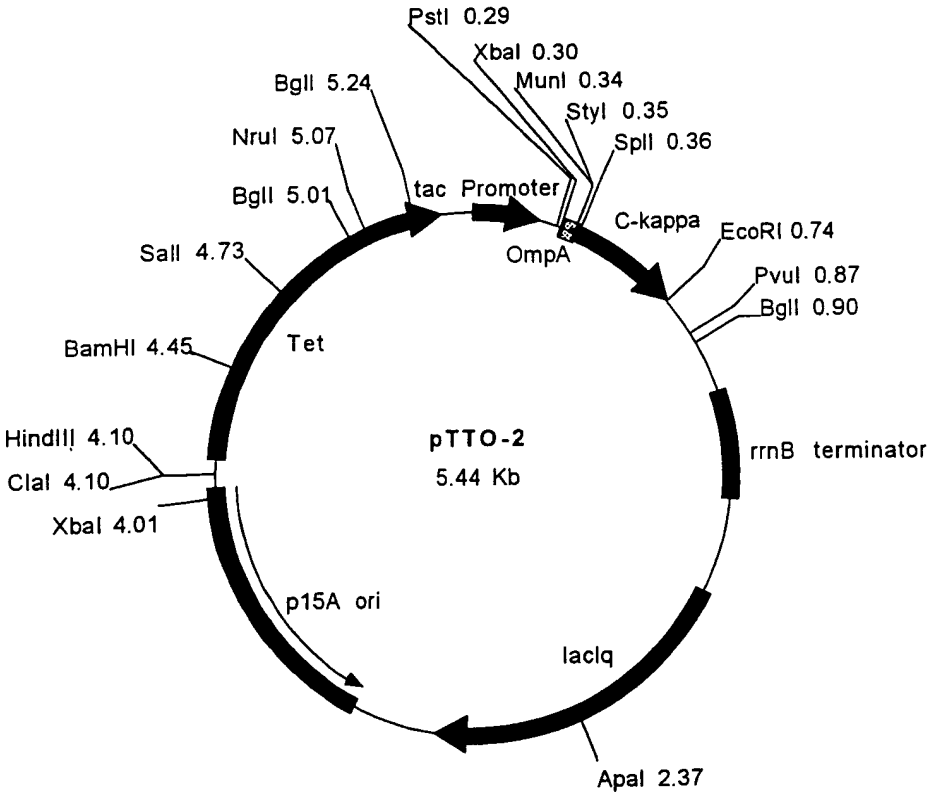

PstI 0.29
XbaI 0.30
MunI 0.34
StyI 0.35
SpII 0.36
BglI 5.24
NruI 5.07
BglI 5.01
tac Promoter
C-kappa
EcoRI 0.74
PvuI 0.87
BglI 0.90
OmpA
SalI 4.73
BamHI 4.45
Tet
HindIII 4.10
ClaI 4.10
XbaI 4.01
pTTO-2
5.44 Kb
rrnB terminator
p15A ori
lacIq
ApaI 2.37

Figure 18

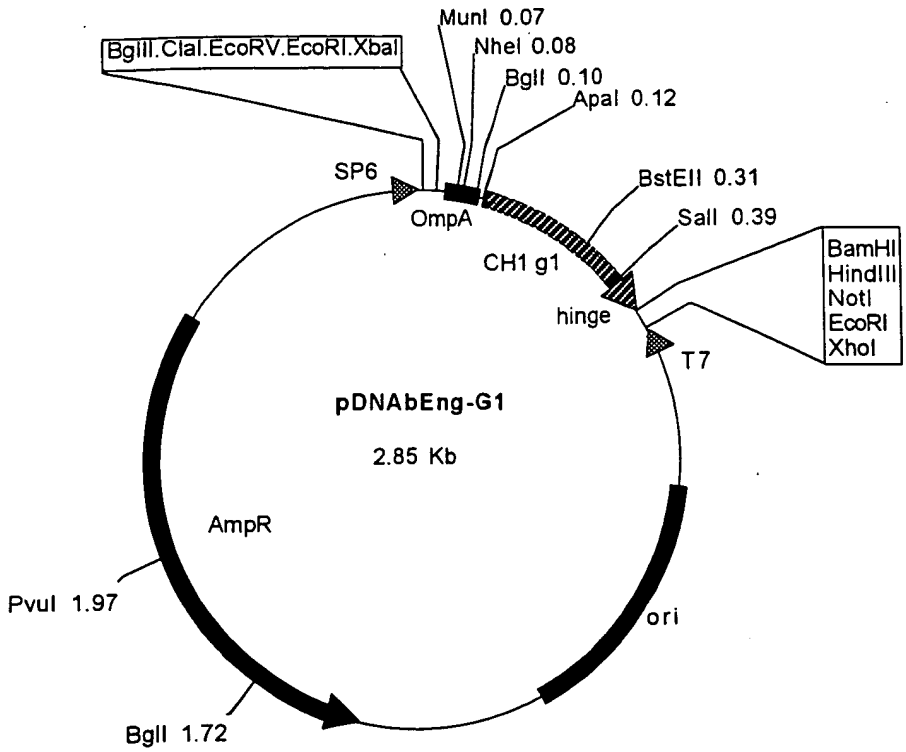

MunI 0.07
NheI 0.08
BglI 0.10
ApaI 0.12
BglII.ClaI.EcoRV.EcoRI.XbaI
SP6
OmpA
BstEII 0.31
SalI 0.39
CH1 g1
BamHI
HindIII
NotI
EcoRI
XhoI
hinge
T7
pDNAbEng-G1
2.85 Kb
AmpR
PvuI 1.97
ori
BglI 1.72

Figure 19

OLIGONUCLEOTIDE CASSETTES ENCODING DIFFERENT INTERGENIC SEQUENCES FOR E. Coli Fab' EXPRESSION

IGS CASSETTE-1; Intergenic space = -1

G,AGC,TCA,CCA,GTA,ACA,AAA,AGT,TTT,AAT,AGA,GGA,GAG,TGT,TAATG,AAG,AAG,ACT,GCT,ATA,GCA,ATT,G

S S P V T K S F N R G E C * M K K T A I A I

End of c-Kappa sequence -> *Start of OmpA sequence ->*

IGS CASSETTE-2; Intergenic space = +1

G,AGC,TCA,CCA,GTA,ACA,AAA,AGT,TTT,AAT,AGA,GGG,GAG,TGT,TAA AATG,AAG,AAG,ACT,GCT,ATA,GCA,ATT,G

S S P V T K S F N R G E C * M K K T A I A I

IGS CASSETTE-3; Intergenic space = +13

G,AGC,TCA,CCA,GTA,ACA,AAA,AGC,TTT,AAT,AGA,GGA,GAG,TGT,TGA GGAGGAAAAAAAAATG,AAG,AAA,ACT,GCT,ATA,GCA,ATT,G

S S P V T K S F N R G E C * M K K T A I A I

IGS CASSETTE-4; Intergenic space = +13

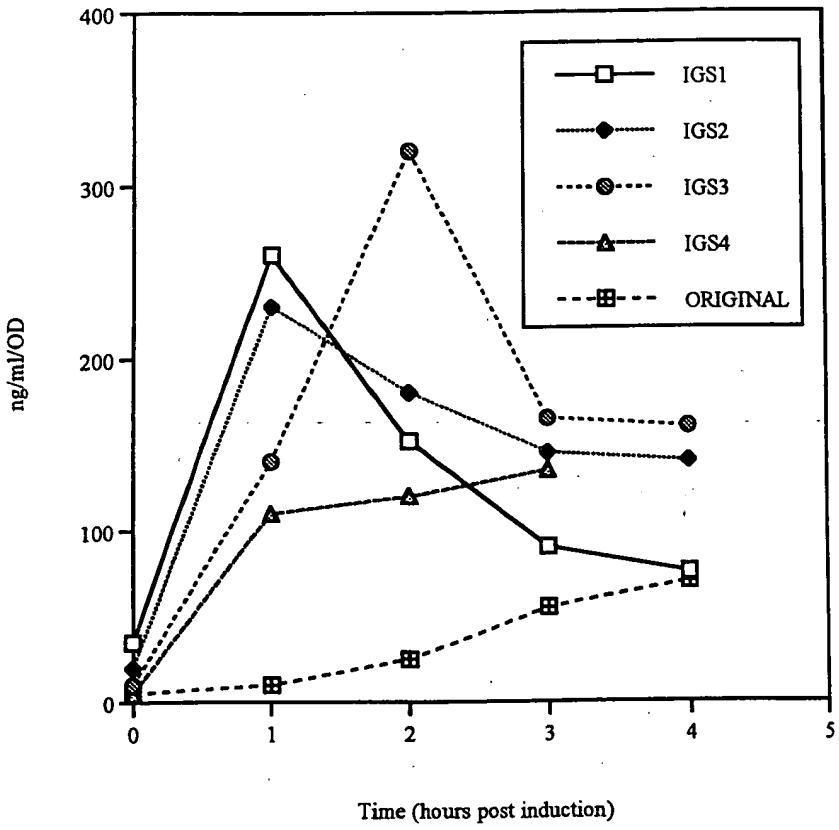
G,AGC,TCA,CCA,GTA,ACA,AAA,AGT,TTT,AAT,AGA,GGA,GAG,TGT,TGA CGAGGATTATATAATG,AAG,AAA,ACT,GCT,ATA,GCA,ATT,G

S S P V T K S F N R G E C * M K K T A I A I

Figure 20

Figure 21

Periplasmic Fab' accumulation - IGS variants



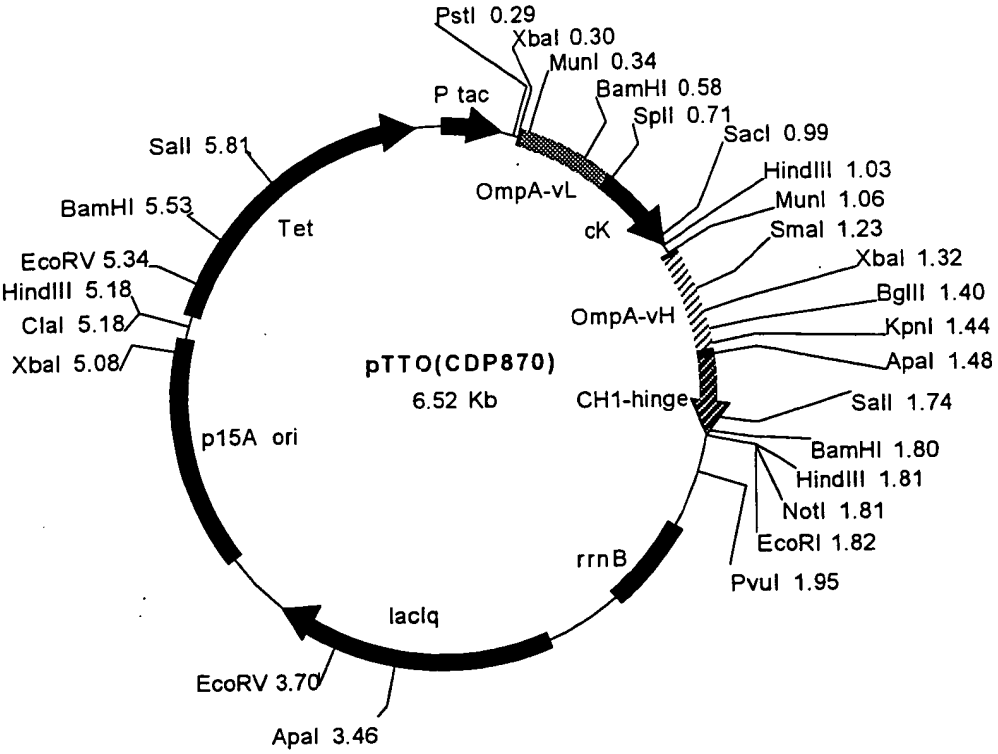

PstI 0.29
XbaI 0.30
MunI 0.34
BamHI 0.58
SpII 0.71
SacI 0.99
HindIII 1.03
MunI 1.06
SmaI 1.23
XbaI 1.32
BglII 1.40
KpnI 1.44
ApaI 1.48
SalI 1.74
BamHI 1.80
HindIII 1.81
NotI 1.81
EcoRI 1.82
PvuI 1.95
rrnB
lacIq
EcoRV 3.70
ApaI 3.46
p15A ori
XbaI 5.08
ClaI 5.18
HindIII 5.18
EcoRV 5.34
BamHI 5.53
SalI 5.81
Tet
P tac
OmpA-vL
cK
OmpA-vH
CH1-hinge
pTTO(CDP870)
6.52 Kb

Figure 22